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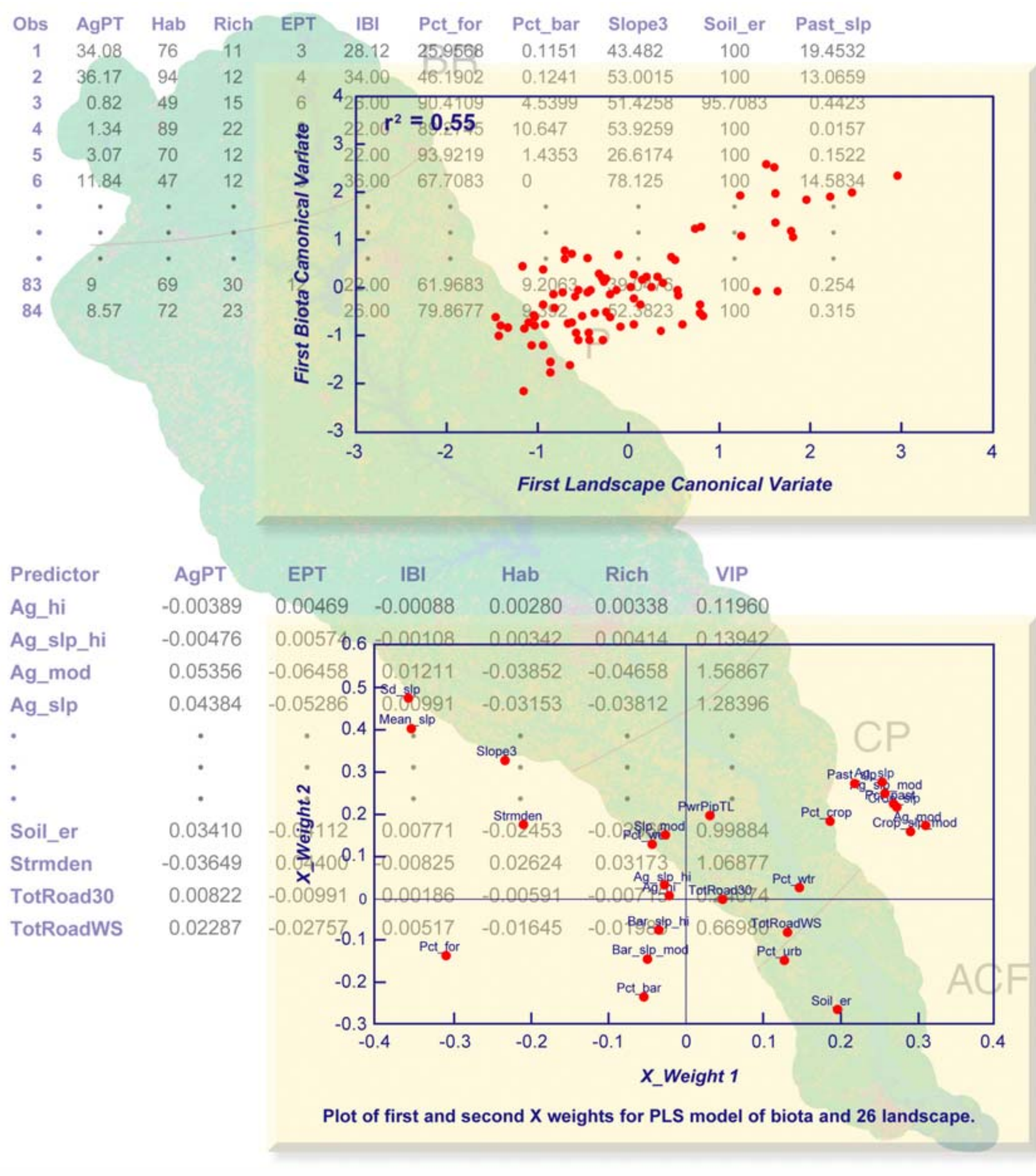
Multivariate Analyses (Canonical Correlation and Partial Least Square (PLS)) to Model and Assess the Association of Landscape Metrics to Surface Water Chemical and Biological Properties Using Savannah River Basin Data



Plot of first and second X weights for PLS model of biota and 26 landscape.

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Multivariate Analyses *(Canonical Correlation and Partial Least Square (PLS))* to Model and Assess the Association of Landscape Metrics to Surface Water Chemical and Biological Properties Using Savannah River Basin Data

by

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Notice

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Section 1

Introduction

In ecological studies, often many measurements are taken from many sites in an area to analyze and explore relationships. A number of statistical methods may be used to analyze and explore relationships among variables. Single- and multiple-regression analysis has frequently been used to relate water nutrient concentrations to selected landscape metrics (Noy-Meir, 1974; Jones et al., 2001; Mehaffey et al., 2001). The above results quantified relationships and answered questions regarding the status of the landscape in an area. But, when the need is to relate two or more distinct data sets (e.g., landscape metrics and chemical properties of surface water) to describe their association and to explain their connection to their physical environment, multivariate analyses, such as canonical correlation and partial least square (PLS) should be used.

Many multivariate methods are used in describing and predicting relation; each has its unique usage of categorical and non-categorical data. In multivariate analysis of variance (MANOVA), many response variables (y's) are related to many independent variables that are categorical (classes, levels). For example, relating nitrogen, phosphorous and fecal coliform to presence/absence of urban development, farm, soil types, geological formations, etc, (nitrogen + phosphorous + fecal coliform = type of farm, urban development, geology, soil, ...). In analysis of variance (ANOVA), a dependent (response) variable is related to many independent variables that are categorical. For example, determining the response of an ant species to grazing level (severe, medium, low) in an area (ant abundance = grazing levels). In multiple discriminant analysis the dependent variable (y) is categorical (groups or classes) and related to the independent variables (x's). For example, presence/absence of amphibians in an area relates to many environmental variables (pres/abs = percent bedrock substrate cover + water depth + percent vegetation cover + ...). In multiple regression the dependent variable (y) is related to many independent variables (x's). For example nitrogen loading relates to landscape metrics such as percent forest, percent crops, percent of wetland, percent of urban development.

In canonical correlation, two sets of variables are related and these variables may or may not be categorical. So it is a generalized multivariate statistical technique in respect to that described above, and is directly related to principal components-type factor analytic models. In canonical analysis method, a number of composite associations between sets of multiple dependent and independent variables are performed. Consequently, a number of independent canonical functions that maximize the correlation between the linear composites of sets of dependent and independent variables are developed. The main goal of the canonical correlation analysis is to develop these linear composites (canonical variate), derive a set of weights for each variate, thereby explaining the nature of relationships that exist between the sets of response and predictor variables that is measured by the relative contribution of each variable to the canonical functions (relationships) that exist. The results of applying canonical correlation is a measure of the strength of the relationship between two sets of multiple variables. This measure is expressed as a canonical correlation coefficient (r) between the two sets.

Canonical correlation analysis is used to describe the association between two sets of variables such as the relationship between water biological metrics as dependent variables, and landscape metrics as independent variables. Canonical correlation analyses results are used to describe association, including vegetation species and environmental conditions (Ter Braak, 1987; Johnson and Altman, 1996). Also canonical correlation analyses results are used to describe the physical process that leads to vegetation variation as a response to environmental conditions.

PLS is also a multivariate analysis well known in chemometrics for use in studying the structure pattern among groups of chemicals. It is the prediction of chemical form from spectroscopy reading, where several hundred wavelengths and a smaller number of chemical samples (Owen, 1988) is the norm in chemometric analyses. PLS is used in Quantification of Molecular Modeling, where a large number of independent variables (>1000) are normally obtained with respect to the number of samples (10 to 100). PLS features, as will be seen below, make it applicable in relating landscape metrics with water quality properties.

Canonical correlation requires a relatively large number of observations compared to the number of variables. It is also sensitive to collinearity in independent variables and requires multinormal data sets. Multinormality is a requirement when the test of the significance for the canonical correlations is considered to define the model rank. In ecology, sample size is often small, number of independent variables is large and the independent variables are frequently correlated. This necessitates excluding important variables from the model. These latter problems are overcome by using PLS. Similar to canonical correlation, PLS outputs many statistics that can be used to describe the variability by the two data sets. But PLS predicts for dependent and independent variables, and also produces the relative importance values of the independent variables. Relative importance values can be used to decide which independent variable contributes the most to the fitted model.

Section 2
Goals and Objectives

Our primary goals were:

1) to determine if there are relationships (dependence) between the two data sets of surface water biota and multiple landscape metrics,
2) to determine if the relationships are significant between the landscape metrics and surface water properties,
3) to quantify the strength of relationships, and
4) to define the key landscape variable(s) that contributes to surface water quality.

Therefore, our objectives were:

1) to explore the relationships among chemical and biological surface water properties and landscape data sets,
2) to quantify the magnitude of the relationship between each of the chemical and biological variables with the landscape metrics, and
3) to investigate the possibility of using chemical data, which is less expensive and easier to measure, as a surrogate for biological data to examine changes in landscape and related effect in water quality metrics.

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Section 3
Data Sets

The water data used in this analysis was provided by EPA Region 4, Science and Ecosystem Support Division. As a Regional Environmental Monitoring and Assessment Program (REMAP) project, site selection and sampling were completed according to standard EMAP protocols. For each of the selected sites, the watershed support area was delineated, and a suite of landscape metrics was calculated (Chaloud, 2001). Three data sets: 1) Water chemical; (2) Water biological, and (3) landscape were used in the analyses (see Table 1 for variable description). Number of Variables entered to the model for canonical correlation were different than that for the PLS, because of normality, missing values and collinearity issues with the canonical correlation (see Appendix I). Variables used for each model are described in each method below.

Table 1. Water chemical-biological and landscape metrics used in the analyses. Letters "c" and "p" donates variables included in canonical and PLS analyses.

Variable Type/Name	Full Name	Description
Water Chemical Metrics		
pH (c)	pH	pH
EC (c)	EC	Electrical Conductivity, micro-cisiemens per centimeter (μS/cm)
DO (c)	DO	Dissolved Oxygen
Water Biological Metrics		
Hab (c,p)	MI_Habitat	Macroinvertebrate habitat.
EPT (c,p)	MI_EPT	Taxa richness of sensitive insects to pollution; EPT stands for Ephemeroptera-Plecoptera-Trichoptera Index. These insects are correlated with good water quality (Lenat, 1987) based on 100-organism subsample, non-impacted (>10), slightly impacted (6-10), moderately impacted (2-5) and severely impacted (0-1).
Rich (c,p)	MI_Richness	Macroinvertebrate richness, species richness (SPP). Total number of species in a sample. Condition of areas are classified as: non impacted (>26), slightly impacted (19-26), moderately impacted (11-18), severely impacted (<11).
AgPT (c,p)	AgPT	Algal growth Potential Test
IBI (c,p)	fish_IBI	Fish Index of Biotic Integrity (Karr, 1981)
Pct_crop (c,p)	Percent crop	Percentage of total MRLC landcover in row crops types
Pct_past (c,p)	Percent pasture	Percentage of total MRLC landcover in pasture/grassland types
Pct_wtr (c,p)	Percent water	Percentage of total MRLC landcover in water types

Table 1 *(Continued)*

Variable Type/Name	Full Name	Description
Landscape Metrics		***Percent area in a HUC***
Pct_wet (c,p)	Percent wetlands	Percentage of total MRLC landcover in wetland types
Pct_bar (c,p)	Percent barren	Percentage of total MRLC landcover in barren types (quarries, strip mines)
Pct_urb (c,p)	Percent urban	Percentage of total MRLC landcover in urban types
Pct_for (c,p)	Percent forest	Percentage of total MRLC landcover in forest types
Crop_slp (c,p)	Crops on slopes > 3%	Percent of total area in row crops on slopes greater than 3 percent
Crop_slp_mod (c,p)	Crops on slopes > 3% and on moderately erodible soils	Percent of total area in row crops on slopes greater than 3 percent and on moderately erodible soils (STATSGO K-factor $ 0.2 and < 0.4)
Ag_hi (c,p)	Agriculture on highly erodible soils	Percent of total area in agriculture (row crops + pasture) on highly erodible soils (STATSGO K-factor $ 0.4)
Ag_slp (c,p)	Agriculture on slopes > 3%	Percent of total area in agriculture (row crops+pasture) on slopes greater than 3 percent
Ag_slp_hi (c,p)	Agriculture on slopes > 3% and on highly erodible soils	Percent of total area in agriculture (row crops + pasture) on slopes greater than 3 percent and on highly erodible soils (STATSGO K-factor $ 0.4)
Ag_mod (c,p)	Agriculture on moderately erodible soils	Percent of total area in agriculture (row crops + pasture) on moderately erodible soils (STATSGO K-factor $ 0.2 and < 0.4)
Ag_slp_mod (c,p)	Agriculture on slopes > 3% and on moderately erodible soils	Percent of total area in agriculture (row crops + pasture) on slopes greater than 3 percent and on moderately erodible soils (STATSGO K-factor $ 0.2 and < 0.4)
Past_slp (c,p)	Ag_slp - crop_slp	Hay pasture on slopes greater than 3 percent
Bar_slp_hi (c,p)	Barren on slopes > 3% and highly erodible soils	Percent of total area in barren cover types on slopes greater than 3 percent and on highly erodible soils (STATSGO K-factor $ 0.4)
Bar_slp_mod (c,p)	Barren on slopes > 3% and on moderately erodible soils	Barren on slopes > 3% and on moderately erodible soils
Soil_er (c,p)	Erodible soils	Percent of total area with highly erodible soils (STATSCO K-factor $0.4)
Mean_slp (c)	Mean slope	Mean or average percent slope
Slope3 (c,p)	Slope >3%	Percent of total area with slopes greater than 3 percent
Slp_mod (c,p)	Moderately erodible soils on slopes > 3%	Percent of total area with moderately erodible soils (STATSGO K-factor $ 0.2 and < 0.4) and slope greater than 3 percent
Sd_slp (c)	Standard deviation slope	Standard deviation of percent slope
Strmden (c,p)	Stream density	Stream density as total length of streams from USGS TIGER data

Table 1 *(Continued)*

Variable Type/Name	Full Name	Description
***Landscape Metrics** (Continued)*		***Percent area in a HUC***
Totroad30 (c,p)	Road30t1 +	Total length of type 1 (interstate) roads within 30 m of streams from USGS TIGER data
	Road30t2 +	Total length of type 2 (interstate) roads within 30 m of streams from USGS TIGER data
	Road30t3 +	Total length of type 3 (interstate) roads within 30 m of streams from USGS TIGER data
	Road30t4 +	Total length of type 4 (interstate) roads within 30 m of streams from USGS TIGER data
	Rail30 +	Total length of railroads within 30 m of streams from USGS TIGER data
	Rr30_siding	Total length of railroad sidings within 30 m of streams from USGS TIGER data
TotroadWS (c,p)	Road_t1 +	Total length of type 1 (interstate) roads from USGS TIGER data
	Road_t2 +	Total length of type 2 (interstate) roads from USGS TIGER data
	Road_t3 +	Total length of type 3 (interstate) roads from USGS TIGER data
	Road_t4 +	Total length of type 4 (interstate) roads from USGS TIGER data
	Road_t0	Total length of type 0 (trails) roads from USGS TIGER data
PwrPipTl (c,p)	Powerline30 +	Total length of power lines within 30 m of streams from USGS TIGER data
	Pipeline30 +	Total length of pipe lines within 30 m of streams from USGS TIGER data
	Telephone30	Total length of telephone lines from USGS TIGER data

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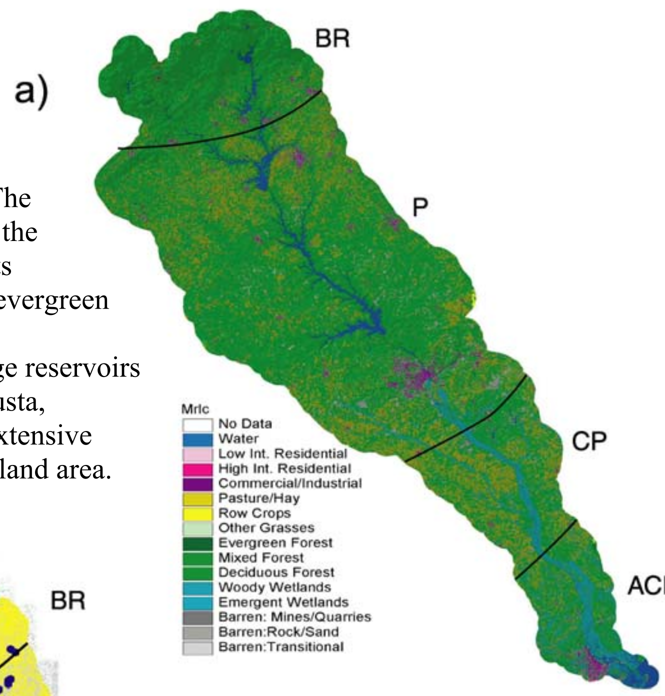
Section 4

Study Site Description

The Multi Resolution Land Characteristics Consortium (MRLC) land cover/land use data for Savannah River Basin (Figure 1a) reveals distinctive spatial patterns. The headwaters of the Savannah River are located in the Blue Ridge Mountains in which evergreen forests predominate. Below this lies a region of mixed evergreen and deciduous forest, agriculture dominated by pasture/hay, and several urban centers. Two large reservoirs can be seen on the main stem river. Below Augusta, Georgia (the large urban center in the middle), extensive row crop agriculture is evident, along with a wetland area. The city of Savannah can be seen near the outlet of the river to the Atlantic Ocean. The spatial patterns seen in the land cover correspond closely to the four ecoregions: Blue Ridge (BR), Piedmont (P), Coastal Plain (CP), and Atlantic Coastal Plain (ACF) (Figure 1b). For this report, only three ecoregions (BR, P and CP) were used.



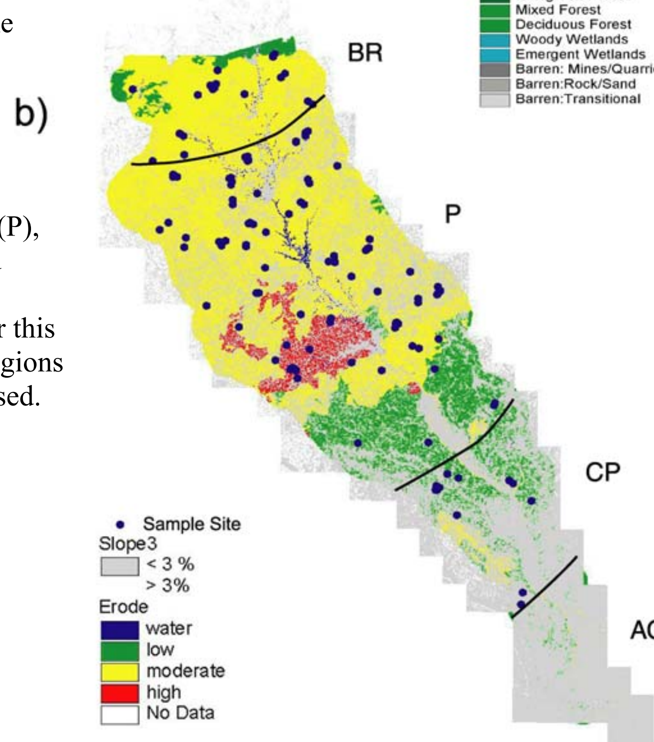


Figure 1. Savannah River Basin (a) ecoregion and MRLC classification, and (b) sample locations and erodibility classes.

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Section 5
Statistical Analyses

Before presenting the canonical correlation analyses, detailed discussions about missing observations, collinearity, normality and others are given in Appendix I for readers. We used SAS for all the statistical analyses listed below.

5.1 Canonical Correlation Analysis

In canonical correlation analysis, first attempt is to derive a linear combination (*canonical variate*) of the variables of each data set so that their correlation would be maximized and would be at least as larger as the multiple correlation between any variable in one data set and all those from the other data set. For example, when water biological properties and landscape metrics were analyzed, the biological and landscape original variables would be combined in a linear relationship, such as:

$$\text{Bio1} = 3 n_i B_i$$

$$\text{Land1} = 3 o_i LS_i$$

Where Bio1 and Land1 are the first pair of biological and landscape canonical variates, ni and oi are the coefficients (sometime referred to as weights) for each of the biological and landscape variables. B and LS are the biological and landscape original variables. Values of the coefficients, ni and oi, are determined so that the correlation between Bio1 and Land1 is maximized. The first pair of canonical variates always has the highest canonical correlation (r_k). The canonical correlation is considered as a multiple correlation of, for example, Bio1 with the landscape metrics, or, Land1 with the biota metrics. At this point, note that each variate (e.g., Bio1) can be considered a principal component (Gittens, 1985), and the covariance between the two variates (e.g., Bio1 and Land1) is maximum. If the canonical correlation is done on the standardized variables, each canonical variate is a principal component and maximizing correlation and covariance are the same.

The analysis produces a second pair (Bio2 and Land2) of linear combination of the original variables unlike the first pair, and this pair of variates has the second highest canonical correlation. The analyses will continue to produce a number of pairs (*k*) until they are equal to the number of variables in the smaller data set. In other words, if the biological data set contains four variables and the landscape data set contains seven variables, then four (*k*) pairs of variates will be produced. They are then sorted by their canonical correlation values, the first being the highest. In some references, *k* is referred to as the rank of the model. For simplicity and clarity, we will use the names below for each pair of canonical variates:

Bio1: first canonical biological variate,

Bio2: second canonical biological variate,

Chem1: first canonical chemical variate, Chem2: second canonical chemical variate,

Land1: first canonical landscape variate, and

Land2: second canonical landscape variate.

In addition to canonical correlation, the analysis outputs other statistics that can be used to fully understand and describe association. These are canonical variate, square canonical correlation, canonical coefficient, and inter-set and intra-set structure correlation. Theory and development of canonical correlation are well stated in many references (Johnson and Wichern, 2002; Rencher, 1998; Hair et al., 1987; Gittins, 1985; Thorndike, 1978; Clark, 1975). A brief description of each output is given below.

5.1.1 Canonical Variate and Model Rank

The number of pairs (*k*) of *canonical variate* is equal to the number of variables in the smaller data set. SAS outputs the canonical correlation value and its significance level for each pair so the number of pairs to be used for interpretations in the final model can be selected. The number of significant pairs in a model is known as the rank of the model. Plotting the scores of variates in a pair describes visually the correlation between the linear combination of the two data sets (variates).

5.1.2 Squared Canonical Correlation

Squared canonical correlation (r^2_k) is also known as eigenvalue or canonical root (Hair et al., 1987). The value of r^2_k measures the proportion of variance of a canonical variate (e.g., Bio1) explained by the original variables of landscape metrics. The value of r^2_k can be considered the multiple regression correlation between the two variates (e.g., Bio1 and Land1; Griffith and Amrhein, 1997) which measures the adequacy of the overall fitted model (Gittens, 1985). The summation of r^2_k over k (the number of canonical pairs) is the trace which measures the amount of variance that is shared and predicted by the two data sets. The final fitted model contains only the significant number (*k*) of canonical correlation. The quality of model is measured by the percent trace (% Trace = r^2_k*100/3r^2_k) and the percent cumulative trace (Table 2).

Table 2. Canonical correlation (r_k), canonical r-square (r^2_k), percentage trace (% Trace = r^2_k*100/3r^2_k) and cumulative trace (%) of total (standardized) sample variance explained by each canonical variate and the probability of exceeding the critical value (F).

a) Biological and Landscape Metrics					
k	**r_k**	**r^2_k**	**Trace %**	**Trace Cum. (%)**	**P>F**
1	0.739	0.546	59.21	59.21	<0.0001
2	0.534	0.286	30.97	90.18	0.0008
3	0.267	0.071	7.71	97.89	0.2953
4	0.139	0.019	2.11	100.00	0.4653

Table 2 *(Continued)*

b) Chemical and Landscape Metrics					
k	**r_k**	**r^2_k**	**Trace %**	**Trace Cum. (%)**	**P>F**
1	0.747	0.559	72.73	72.73	<0.0001
2	0.378	0.143	18.56	91.30	0.0134
3	0.259	0.067	8.70	100.00	0.0827

c) Biological and Chemical Metrics					
k	**r_k**	**r^2_k**	**Trace %**	**Trace Cum. (%)**	**P>F**
1	0.543	0.353	59.62	59.62	<0.0001
2	0.480	0.231	39.01	98.62	0.0034
3	0.090	0.008	0.38	100.00	0.7451

5.1.3 Canonical Coefficients

Canonical coefficients are also known as canonical loadings or weights, and each canonical variate is a linear combination of the original variables weighted by their coefficients. The eigen vector associated with the (k^{th}) eigen value gives the weights for combining one set on variables (dependent). Weights for other set of variables is produced by substituting these into equation and solving. Hence, loading measures the contribution of each of the original variables to the canonical variate (see coefficients in equations for the canonical variates on page 19). These weights were estimated so that the correlation between the two variates in a pair is maximized. Therefore, the values of these weights are expected to be different in various samples. For interpretation of results, standard canonical coefficients (not raw canonical coefficient) were used for unification of units and scales of the original variables. To quantify the contribution of each variable to the canonical variates in a multivariate context, Rencher (1998) and Johnson and Wichern (2002) recommended using the standardized coefficients instead of the correlation between the original variable and canonical variable. For example, the coefficients ni's (Bio1 = 3ni Bi) reflect the joint contribution of the biota variables to the correlation between Biota1 and Land1.

An important note to make is that when the sample size is small and the ratio of number of variables to the number of observations is high, then the coefficients are unstable. More discussion about this issue is included in Appendix I.

5.1.4 Correlation Between the Original Variables and Canonical Variates

This is known as *Intra-set structure correlation*. The intra-set correlation describes the amount of variance that is contributed by the original variable to the canonical variate. (If the intra-set correlation is squared, it will give the amount of variance that is explained by its variate). Intra-set correlation indicates the strength of the association between the original variable and the canonical variates (which should give an indicator of the importance of the contribution of the original variable to the canonical variate). The square of the intra-set correlation would give the proportion of the variance of the canonical variate that is explained by the original variable.

The correlation between each of the original variables and the opposite canonical variate is known as *Inter-set structure correlation*. This correlation is analogous to that of multiple correlation coefficients that describe the linear relationship between the original variable and the opposite canonical variate. For example, index of biological integrity (IBI) can be correlated to the water chemistry canonical variate. When the inter-set correlation value is squared, it will measure the amount (proportion) of variability in the original variable that is predicted by the opposite canonical variate. It is analogous to partial R^2 value. SAS outputs the latter at the last part of SAS canonical correlation analyses “squared multiple correlation in redundancy analysis” (see Table 3).

Table 3. Canonical correlation structure: correlation between the original variables and the canonical variate.

a) Biological and Landscape Metrics

Variable	Bio1	Bio2	Land1	Land2
AgPT	-0.433	0.221	-0.320	0.118
IBI	-0.324	0.267	-0.239	0.143
Hab	0.211	-0.816	0.156	-0.436
Rich	0.431	-0.067	0.319	-0.036
EPT	0.856	0.052	0.633	0.028

Variable	Land1	Land2	Bio1	Bio2
Slope3	0.831	0.521	0.614	0.279
Soil_er	-0.151	0.884	-0.112	0.472
Pct_bar	-0.109	-0.556	-0.081	-0.297
Past_slp	-0.321	-0.669	-0.238	0.358

b) Chemical and Landscape Metrics

Variable	Chem1	Chem2	Land1	Land2
DO	0.891	0.078	0.666	0.0293
pH	-0.120	0.949	-0.090	0.358
EC	-0.844	0.037	-0.631	0.014

Variable	Land1	Land2	Chem1	Chem2
Pct_bar	-0.524	0.211	-0.392	0.080
Pct_for	0.073	0.871	0.054	0.329
Past_slp	0.398	-0.249	0.297	-0.094
Slope3	0.872	0.472	0.652	0.178

c) Chemical and Biological Metrics

Variable	Bio1	Bio2	Chem1	Chem2
IBI	0.111	0.580	0.066	0.279
Hab	0.254	0.053	0.151	0.025
Rich	0.617	0.576	0.366	0.279
EPT	0.901	0.065	0.535	0.031

Variable	Chem1	Chem2	Bio1	Bio2
DO	0.714	-0.575	0.424	-0.276
pH	0.339	0.721	0.201	0.346
EC	-0.736	0.336	-0.437	0.162

The correlation between each of the original variables and the canonical variates is more stable than the row or standardized canonical weights (Gittens, 1985). Many recommended, therefore, using correlations instead of coefficient. Rencher (1998), however, verified the un-stability of coefficient when a variable is added or deleted because it is a reflection of the mutual influence (multivariate) between variables in a canonical variate. The correlation between the original variable and its variate provide no information about the multivariate contribution of a variable to its variate. The relationship described by this correlation is a univariate relationship between the dependent and independent variables (Rencher, 1988, 1998, page 329). The square value of the correlation will measure the multiple correlation of that variable (e.g., IBI) with the other set of variables (e.g., landscape variables). Therefore, correlations will not describe the joint contribution of the biota (dependent variables) to the canonical correlation with the landscape metrics (independent variables).

Correlations between the original variables and its variate and redundancy analyses are normally used to describe contribution of the original variable to its or opposite canonical variate. With the above discussion, these statistics will not provide information in a multivariate relationship. Canonical correlations, square canonical correlations, and percent trace (Table 2) are the measures to be used to describe the multivariate association between the y's and x's.

5.1.5 Variables Entering the Canonical Correlation Analysis

Issues of missing values, collinearity and others are described in detail in Appendix I. These issues may be of concern when applying canonical correlation analyses because of their possible effect on the number of observations and variables to be used in the analyses. In addition to the above issues, we eliminated any *nested structure* in a group of watersheds to eliminate a confounding effect. Sampling stations that were within the watershed support area of a downstream sampling station were eliminated from analysis. The final data set for the canonical correlation consisted of (for variable descriptions see Table 1):

1) Eighty-four sample sites with five biological (AgPT, IBI, Hab, Rich, EPT) and four landscape (Past_slp, Pct_bar, Soil_er, Slope3) metrics,
2) Seventy-seven sample sites with three chemical (pH, DO and EC) and four landscape (Pct_for, Pct_bar, Past_slp, Slope3) metrics, and
3) Seventy-seven sample sites with three chemical (pH, DO, EC) and four biological (IBI, Hab, Rich, EPT) metrics.

5.1.6 Results

Pairwise Correlation of the Original Variables

a) **Biological and Landscape Metrics:** The correlation for the biological data was highest for the EPT and Rich (0.82), whereas, for landscape metrics it was 0.43 for Pct_bar and Past_slp. The highest correlation between the biological and landscape metrics was between EPT and Slope3 (0.54).

b) **Chemical and Landscape Metrics:** The absolute values of correlation for the chemical parameter was highest for the DO and EC (0.51). A negative correlation was observed between DO and Pct_bar (-0.42), and between EC and Slope3 (-0.56). Positive correlation was found between pH and Pct_for (0.33). However, the highest correlation between the chemical and landscape metrics was between DO and Slope3 (0.58).

c) **Chemical and Biological Metrics:** The highest correlation between the chemical and biological parameters was between EC and EPT (-0.41) followed by DO and EPT (0.349).

Canonical Correlation

a) **Biological and Landscape Metrics**: The canonical correlation values (r_k) are reported in Table 2a, in which the first two canonical variates were significant (p # 0.0008). Biological-landscape fitted model, therefore, consists of the first canonical (Bio1-Land1), and second (Bio2-Land2) variates. The linear relationship between the first two canonical variates can be visually examined in the scatter plot of the scores of the first (r=0.74) and second (r=0.53) canonical variates (Figures 2a,b). Squared canonical correlations (r^2_k; Table 2a) express:

1) the variation in the linear combination of the biological metrics that is attributable in the linear combination of the landscape metrics,
2) the proportion of the variance of Bio1 explained by the original variables of landscape metrics, and

3) the amount of shared variance between water biology and landscape original variables.

The first, and second canonical variate explains 0.55 and 0.29, respectively, of the variation as compared to less than 0.09 for any of the remaining canonical variates. The sum of r^2_k (=0.92; Table 2a) measures the variance shared by all four pairs of canonical variates. The fitted model, which represents only the significant pairs (first, and second), accounted for 90% (%Trace = (0.55+0.29)*100/0.92; Table 2a) of that overall shared variance. Percent trace can be used as an index of the fitted model quality that measures prediction adequacy.

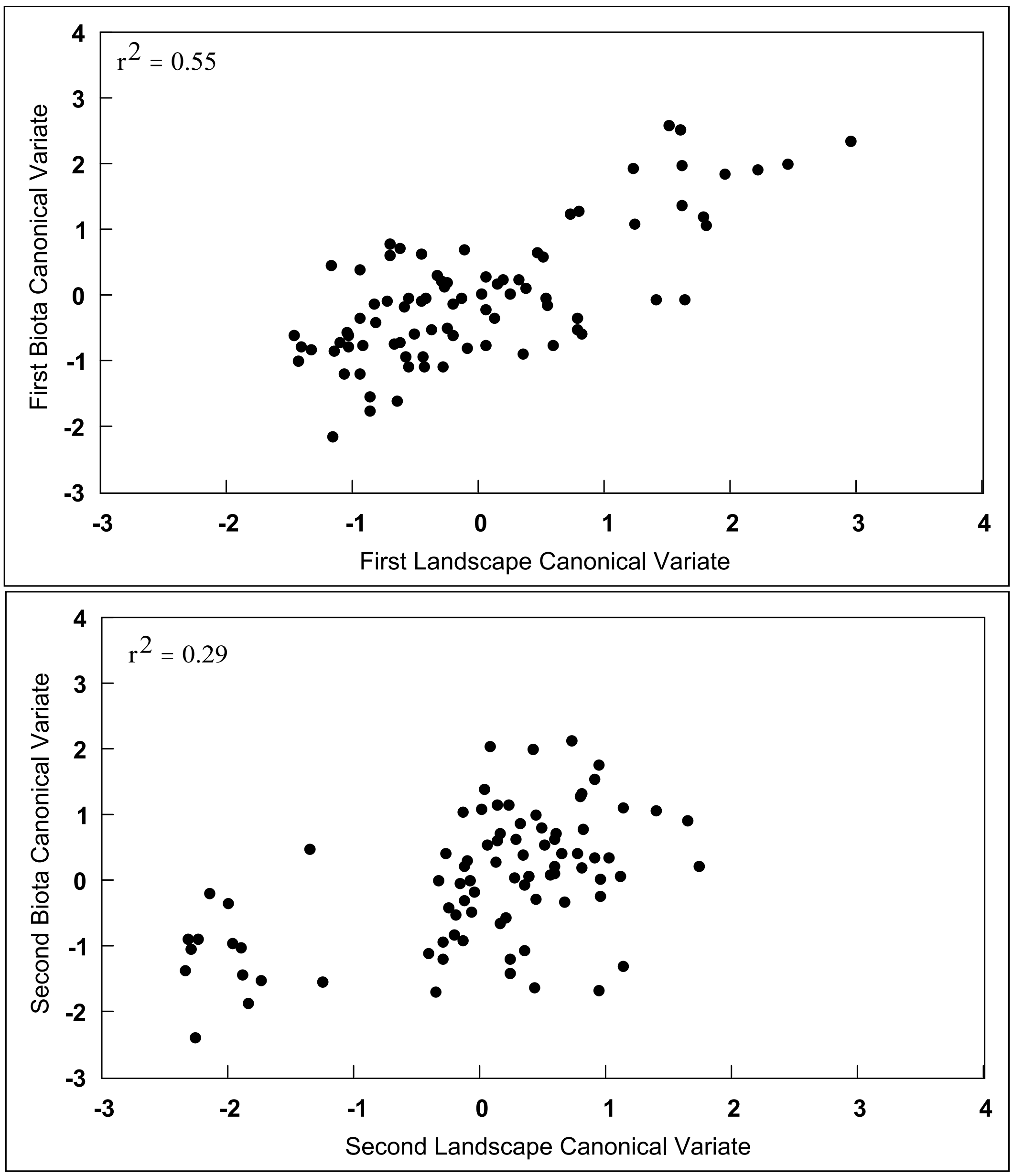

Figure 2. The scatter plot of the first two pairs of canonical variates for the landscape (Past_slp, Soil_er, Pct_bar and Slope3) and water biota (AgPT, IBI, Hab, Rich, EPT).

b) Chemical and Landscape Metrics: The first two canonical correlations are significant (P # 0.0134; Table 2b). The fitted model consists of the first two canonical variates only. The strength of the linear relationships for the first two pairs are 0.75 and 0.38, respectively. The percent variation of a canonical variate of water chemistry attributable to that of a landscape was 0.56 and 0.14 for the first and second pairs. Variance shared by all three pairs was 0.768 (the sum of the r^2_k in Table 2b). Our fitted model accounted for 91% (%Cum. Trace = (0.56+0.14) *100/0.768)) of the variance that is shared by all canonical variates. The quality index (91%) of the fitted model indicates that chem1 and chem2 are adequately predicted water chemical parameters derived from landscape metrics (Land1 and Land2). The linear correlation between the chemical and landscape metrics for the canonical variates is shown in Figure 3.

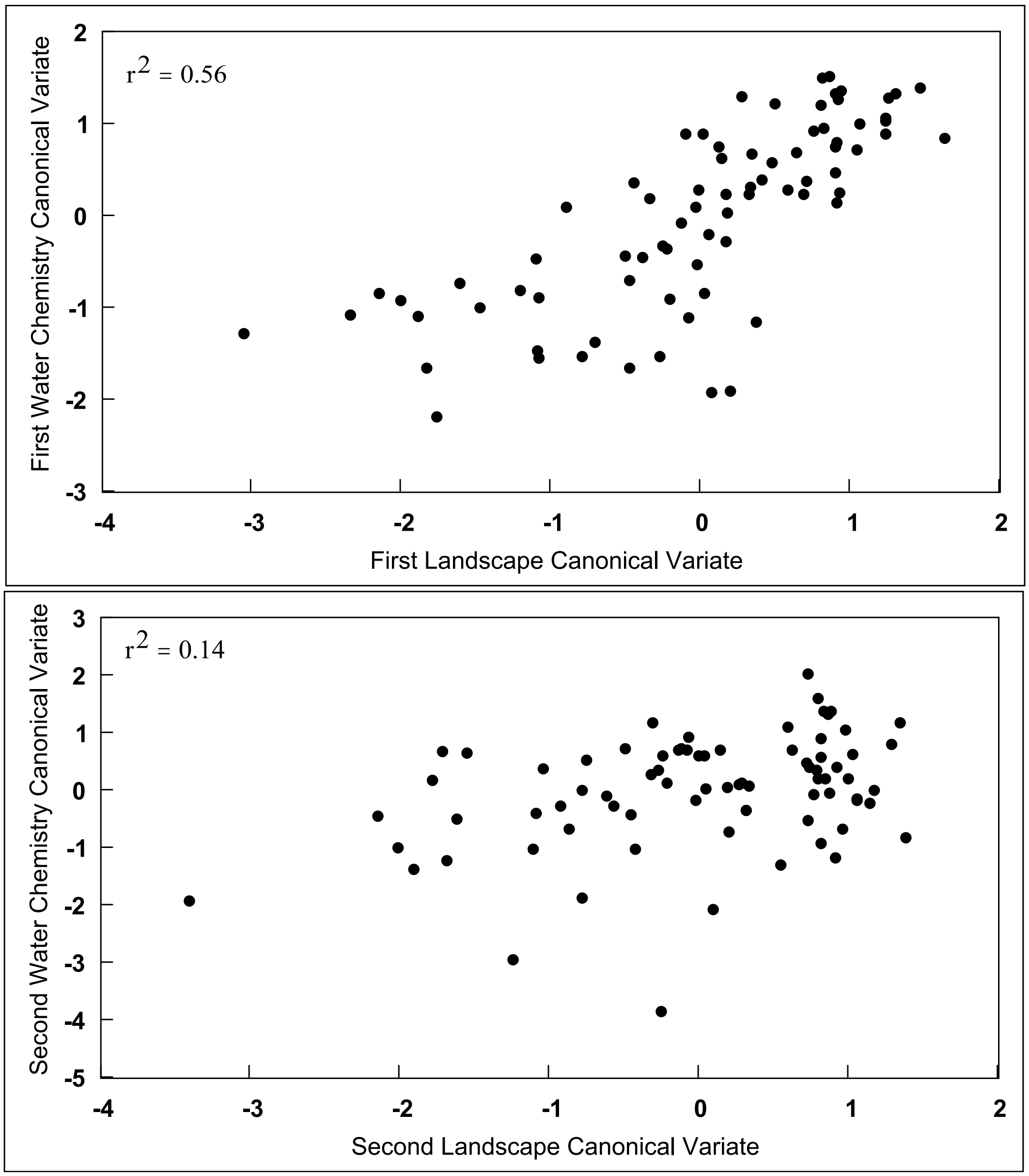

Figure 3. The scatter plots of the first two pairs of canonical variates for the landscape metrics (Past_slp, Pct_for, Pct_bar, and Slope3) and water chemistry (DO, pH, and EC).

c) Biological and Chemical Metrics: The first two canonical correlations are significant (P # 0.0034; Table 2c). Our fitted model consists of Bio1-Chem1 and Bio2-Chem2. The strength of correlation between Bio1-Chem1 is 0.59, and between Bio2-Chem2 is 0.48. The amount of variation in Bio1 attributable to Chem1 is 0.35, and the amount of variation in Bio2 attributable to Chem2 is 0.23. The variance shared by all three canonical variates is 0.59 (the sum of the r^2_k in Table 2c). Our fitted model accounts for 99% (%Cum. Trace = (0.35 +0.23)*100/0.59) of the variance that is shared by all the canonical variates. The quality index (99%) of the fitted model indicates the high quality of prediction. The linear correlation between the chemical and landscape metrics for the canonical variates is shown in Figure 4.

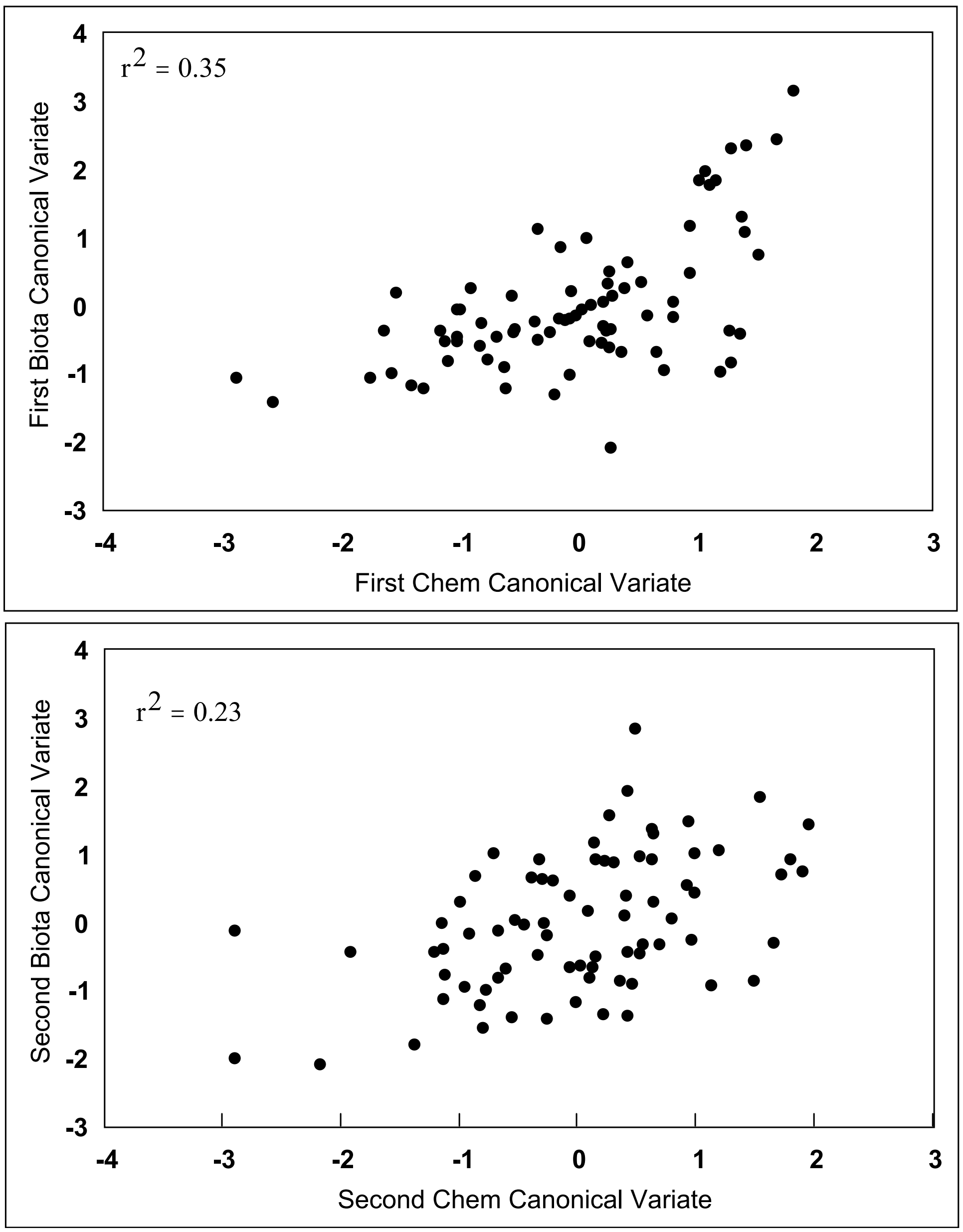

Figure 4. The scatter plots of the first two pairs of canonical variates for the water biota (IBI, Hab, Rich, EPT) and water chemistry (DO, pH, and EC).

Canonical Coefficient

For this section we list only the linear combination of the original variables for variates in the canonical pair(s) that is(are) significant. Coefficient (or weight) indicates the relative contribution of each variable (standardized) to the canonical variate by magnitude and direction (positive or negative; see equations below). A coefficient value reflects the contribution of a variable in presence of other variables that are in their canonical variate. This contribution has its effect on the opposite canonical variate as measured by the canonical correlation. Hence, multivariate context is more evident in coefficients than that in correlation of the original variables with its canonical variate (more discussion in inter- and intra set correlation).

a) **Biological and Landscape Metrics:** The contribution of the EPT and Hab are positive for the first biological canonical variate, and AgPT, IBI and Rich are negative (see equations below). The contribution of the EPT is the highest (1.484). The landscape canonical variate is weighted negatively on Pct_bar, Past_slp, and Soil_er and positively on Slope3. The highest values in magnitude are for the Slope3 (0.954). The first canonical correlation reflects the relationships between the biota variate (EPT and Rich) and landscape variate (Slope3 and Past_slp). When Slope3 increases, it causes an increase in EPT and a decrease in Rich; when Past_slp increases, AgPT increases. The second canonical correlation reflects the relationships between the Hab and Soil_er. When Soil_er increases, it causes a decrease in Hab. Land1 and Land2 represent Slope3 and Soil_er, respectively. Whereas, Bio1 and Bio2 represent EPT/Rich and Hab, respectively.

$$\text{Bio1} = -0.207*\text{AgPT} - 0.008*\text{IBI} - 0.895*\text{Rich} + 0.110*\text{Hab} + 1.484*\text{EPT}$$

$$\text{Land1} = -0.1604*\text{Pct\_bar} - 0.4315*\text{Past\_slp} - 0.337*\text{Soil\_er} + 0.954*\text{Slope3}$$

$$\text{Bio2} = 0.229*\text{AgPT} + 0.488*\text{IBI} - 0.2347*\text{Rich} - 0.942*\text{Hab} + 0.651*\text{EPT}$$

$$\text{Land1} = -0.0814*\text{Pct\_bar} + 0.368*\text{Past\_slp} + 0.654*\text{Soil\_er} + 0.251*\text{Slope3}$$

b) **Chemical and Landscape Metrics:** DO and EC are loaded with approximately the same magnitude on the first canonical chemical variate but with opposite signs (see the equations below). Whereas, the second chemical variate is heavily weighted on pH. The coefficients for the first and second landscape variate are heavily weighted on Slope3 and Pct_for, respectively.

$$\text{Land1} = -0.341*\text{Pct\_bar} - 0.615*\text{Pct\_for} - 0.299*\text{Past\_slp} + 1.130*\text{Slope3}$$

$$\text{Chem1} = 0.634*\text{DO} - 0.032*\text{pH} - 0.511*\text{EC}$$

$$\text{Land2} = 0.415*\text{Pct\_bar} + 1.3702*\text{Pct\_for} + 0.743*\text{Past\_slp} - 0.204*\text{Slope3}$$

$$\text{Chem2} = -0.232*\text{DO} + 1.088*\text{pH} - 0.399*\text{EC}$$

c) **Chemical and Biological Metrics:** Coefficients for Bio1-Bio2 and Chem1-Chem2 are given in the equations below. EPT and Rich are weighted heavily (positive) on Bio1 and Bio2 (1.434, 1.448). EC (-0.810) and pH (0.909) are the main contributors to Chem1 and Chem2, respectively.

$$\text{Bio1} = 0.396*\text{IBI} - 0.564*\text{Rich} + 0.047*\text{Hab} + 1.434*\text{EPT}$$

$$\text{Chem1} = 0.602*\text{pH} + 0.281*\text{DO} - 0.810*\text{EC}$$

$$\text{Bio2} = 0.412*\text{IBI} + 1.448*\text{Rich} - 0.180*\text{Hab} - 0.991*\text{EPT}$$

$$\text{Chem2} = 0.909*\text{pH} - 0.820*\text{DO} - 0.377*\text{EC}$$

Examining the signs of the coefficient in equations above, signs are switched sometimes between the first and second for that canonical variate. This is done to maximize correlation between that pair of canonical variate.

Canonical Structure (Canonical Redundancy Analyses)

Correlation of each original variable with its canonical variate or its opposite. The values below describe the relation between the original variable and its or opposite canonical variate. These correlations do not reflect the multivariate relationships. That is, a correlation value describes a univariate relation between the a variable and its canonical variate, without considering the existence of other variables. The canonical structure is the most used and cited frequently but its usefulness in multivariate context is the least (Rencher, 1998; Johnson and Wichern, 2002).

Within Each Data Set (Intra-set Correlation):

a) **Biological and Landscape Metrics:** The correlation of each of the original variables with its canonical variate is given in Table 3a. For the first canonical variate, the direction of contribution was positive for the Hab, Rich and EPT, and was negative for the IBI and AgPT (Table 3a). The highest correlation was with the EPT (0.856) followed by AgPT (-0.433) and Rich (0.431). Therefore, the biological canonical variate (Bio1) represents mainly EPT and Rich. The landscape original variables correlate negatively on Land1 except for Slope3 (0.83). Past_slp correlated second to the highest and related negatively to Land1. The canonical landscape variates (Land1 and Land2) explained 66% of the variation in landscape variables. The canonical biota variate (Bio1 and Bio2) explained 41% of the variation in biological variables.

b) **Chemical and Landscape Metrics:** Chem1 and Land1 basically represent the DO, EC and Slope3, respectively (Table 3b). Chem2 and Land2 were loaded mainly by pH and Pct_for, respectively (Table 3b). Chem1 and Land1 denote the common pattern between the two data sets that is contributed by Slope3 and DO. Chem2 and Land2 denote the common pattern between the two data sets that is contributed by Pct_for and pH. The canonical landscape variates (Land1 and Land2) account for 57% of the variation in landscape variables, and the canonical chem variates (Chem1 and Chem2) account for 81% of the variation in water chemistry variables.

c) **Chemical and Biological Metrics:** The direction of correlation was positive for all biological variables with the first canonical biological variate (Bio1, Table 3c). The highest correlation was with EPT (0.90), followed by Rich (0.62). Therefore, the biological canonical variate represents mainly EPT and Rich. Bio2 represents mainly IBI and Rich. Rich is common in both Bio1 and Bio2 and were correlated positively with Bio2. The original chemical variables correlated positively, except for EC (-0.74) and DO (-0.58), with Chem1 and Chem2, respectively (Table 3c). The canonical Bio variates (Bio1 and Bio2) account for 49% of the variation in bio variables, and the canonical chem variates (Chem1 and Chem2) account for 71% of the variation in water chemistry variables.

One important point to make here is the discrepancy in sign for some coefficients in a variate (page 19) and in the correlation (Table 3). For example, the coefficient values for the IBI and Rich (page 19, Landscape-Biological) have opposite values than for correlation (Table 3a). This is normally explained by the collinearity between variables. The canonical correlation included variables with pairwise correlation not to exceed 0.9. The highest correlation was 0.82 (EPT and Rich) and all other correlations were less or equal to 0.54.

Between Data Sets (Inter-set Correlation):

a) **Biological and Landscape Metrics:** The direction and the strength of the correlation of each of the original biological variables with the opposite canonical variate were different. EPT, Hab, and Rich were positively correlated with Land1 (0.633, 0.319, and 0.156), whereas, AgPT and IBI were negatively correlated with Land1 (-0.32, and -0.239;Table 3a). As pointed out earlier, Land1 was heavily weighted by Slope3. Hence, EPT, Rich and Hab are related directly to the total watershed area with slopes greater than 3 percent. A higher value in Slope3 (percent total area regardless of landcover type) will enhance EPT and Rich in surface water; however, Past_slp negates them. The first two canonical landscape variates (Land1 and Land2) explain 18% variation in first two canonical biota variates. The first two canonical biota variates (Bio1 and Bio2) explain 24% variation in first two canonical landscape variates. Amount of variability explained by the opposite canonical variate is low, indicating that both Land and Bio variates are not good overall predictors of the other set of original variables.

b) **Chemical and Landscape Metrics:** Chemical parameters responded differently with changes in landscape. The correlation between the original chemical variables with both Land1 and Land2 (Table 3b) indicated that there is a direct positive response of DO to the changes in Landscape, and it had the opposite response for pH and EC. The strongest relationship was for DO (0.67). The magnitude of the correlation of DO with the Land1 is basically the response of DO to the topographical feature (Slope3). A high Pct_bar caused higher EC (Land1 and Chem1; Table 3b). All chemical variables were found to correlate positively with the Land2, with the highest value being for the pH. The direction and magnitude of the correlation in Chem2 and Land2 indicate that high water pH relates to a high Pct_for and lower Past_slp. The canonical landscape variates (Land1 and Land2) explained 33% variation in water chemistry.

c) **Chemical and Biological Metrics:** The relationship between water chemical and biological parameters is of interest to this study. The strength of the relationship may reveal the adequacy of the chemical data as a surrogate to biological data, which will be cost effective for future studies. The correlation between the chemical variable with both Bio1 and Bio2 (Table 3c) indicates that there was a direct positive response of all biological data with Chem1 and Chem2. The strongest relationship was found for the EPT and Rich (0.535 and 0.366) on Chem1, respectively. Chemical variables EC and DO correlate negatively with Bio1 and Bio2, respectively. The magnitude of the correlation of EC and DO with Bio1 and Bio2 (Table 3c) basically influences the lower abundance of the EPT and Rich. The canonical Chemistry variates (Chem1 and Chem2) explain 15% variation of the biota.

R^2 and Canonical Redundancy Analysis

When the canonical correlation between each of the original variables and the opposite variate (e.g., EPT and Land1; Table 3a) is squared, the results are known as the squared multiple correlation or R^2. For example, the square of 0.633 (correlation of EPT with Land1; Table 3) is 0.4002. That is, 40% of the variability in EPT alone was explained by the landscape variate. The squared multiple correlation indicate that the first canonical variable of the biota has more predictive power for EPT (0.40), Rich (0.105) and AgPT (0.1025) than that for IBI (0.057) and Hab (0.024). Whereas, the second biota variate has more predictive power for the EPT (0.401) and Hab (0.215). The first canonical variate of Land is a fairly good predictor of Slope 3 (0.377), poorer predictor for Soil_er (0.0125) and Past_slp (0.0564) and nearly useless for predicting Pct_bar (0.0065). The second canonical variate of Land is fairly predictor of slope 3 (0.455), Soil_er (0.2354), and Past_slp (0.1843) and is nearly useless for predicting Pct_bar (0.009).

For chemistry and landscape data sets, the first and second landscape canonical variates are fairly good predictors of DO ($R^2 = 0.44$) and EC ($R^2 = 0.40$).

For biota and chemistry data sets, the first and second chemical canonical variates are poor predictors of EPT ($R^2 = 0.29$) and Rich ($R^2 = 0.21$).

Partial R^2 values can be found in the redundancy test SAS-output.

Again, Rencher (1998) proved that R^2 values do not provide information in multivariate context but rather in univariate.

5.1.7 Summary

Applying canonical correlation above resulted in a measure of the strength of the relationship expressed by the canonical correlation (r) between two sets of multiple variables, biological and landscape variables. If the canonical correlation is significant, it would indicate the existence of relationships between the two sets and these data sets are not independent. Coefficients or weights for each set of dependent (e.g., biota) and independent (landscape) variables in canonical variate were determined to maximize the correlation between the linear combinations. By applying canonical analysis, it is possible therefore, to develop a number of independent canonical variates that maximize the correlation between the linear composites of sets of biota and landscape metrics.

Statistics from SAS outputs can easily be used to describe and understand the relationships. Many references cautioned when using some of these statistics, such as the coefficients or weights (Rencher, 1998; Hair et al., 1987; Gittens, 1985), because they are sensitive to the variability between observations. Others recommended using the inter-set and intra-set correlations to describe the variance shared by the two data sets. We made the effort to use and describe all the statistics to show the pattern of relationships between landscape and water chemical and biological properties.

Within the traditional inception in fitting any regression model, high R^2 value is the sign of a successful and well received research paper. Many models may fit the data well (Hair et al., 1987), but have a low R^2 value. Other statistics, as an alternative, may give a better presentation. Field data, especially environmental, rarely have a high R^2 value, yet the fitted model may describe a physical phenomenon and a pattern of relationships to other variables. When an attempt was made to describe the amount of variability of biota that was explained by the landscape metrics, R^2 value was not high. However, the canonical correlation analyses for these data sets revealed a useful pattern of relationships between landscape and surface water quality. High correlations between the variates, the quality index of the fitted model, standardized coefficient of the original variables in the canonical variate, and the correlation between the original variables with their own or opposite canonical variates helped to reveal an interesting pattern of relationships. Canonical correlation was used here for exploring relationships and not for predictive purposes.

The landscape-biota model indicated three major contributing variables: the Land variable slope greater than 3 percent (Slope3), the Bio variable EPT (an indicator of three microinvertebrate genera), and the Bio variable Rich (an index of microinvertebrate species richness). Within this model, the Land variable pasture on slopes greater than 3 percent was the second highest landscape contributor, with a negative relationship to the Bio variables. Slopes greater than 3 percent was also the major contributing landscape metric in the landscape-chemistry model; the major Chem contributing variable in this model was dissolved oxygen (DO). In the chemistry-biota model, EPT and Rich were again the major contributing Bio variables, while EC and pH were the major contributing Chem variables, with EC negatively related to biota.

The strength of landscape and chemistry relationships were higher than that of landscape and biota. In circumstances where biota data are unavailable or cannot be measured because of costs or other restrictions, the condition of the surface water biological properties can be assessed using chemistry data as surrogates.

In conclusion, canonical correlation analyses indicated increased slope (indicating complex topography, generally occurring in the mountainous areas of the Savannah River Basin) is associated with increased microinvertebrate quality and higher DO concentrations, while the percentage of landcover in pasture on slopes greater than 3 percent is associated with increased conductivity and declines in aquatic biota quality.

5.2 Partial Least Square (PLS) Analysis

This method is widely used in chemometrics for quantitative structure property relationships research to describe how structural variations in chemical compounds affects biological activity. It is the prediction of chemical form from spectroscopy reading, where several hundred wavelengths and a smaller number of chemical samples (Owen, 1988) is the norm in chemometric analyses. In Quantification of Molecular Modeling, a large number of independent variables (>1000) are normally obtained with respect to the number of samples (10 to 100).

In ecology, we are interested in describing how the structural variation in landscape metrics may affect surface water biological and chemical properties. PLS can be of use in landscape studies utilizing the variation in both independent and dependent data sets. With the advances in GIS technology, obtaining landscape metrics for the past and present in a study area is no obstacle. This may or may not be, especially for the past, the case in obtaining the same amount of surface water data. Past data for surface water quality may either not be complete (missing/nonmeasured) or be a small sample size. Hence, PLS is a suitable method to use to describe and compare relationships over time.

PLS projections of latent structures is a multivariate analysis, and it is specifically close to canonical correlation. Also, it is a generalization of multiple regression. In contrast to other multivariate analyses (e.g., canonical correlation analysis), PLS is a linear predictive model for the dependent and independent data sets. In multiple regression, the variation in independent variables is used to predict the dependent variable. PLS, on other hand, uses the variation in both independent and dependent data sets to predict for the dependent variables. In multiple regression, as well as in canonical correlation, a strong collinearity in independent variables and a large number of independent variables compared to that of observations are potential problems and have to be dealt with before running the analyses. In addition, both multivariate and multiple regression cannot handle missing data. That is, an observation will not be included in analyses when a value is missing for any variable, either dependent or independent. PLS, however, can analyze data sets with missing values (more discussion below), collinear independent variables, or independent variables that have no structure in their behavior ("noisy"), and can also predict for the dependent variables.

PLS analyzes two data sets (e.g., biological and landscape data sets). Both data sets are first centered and scaled (e.g., Bio0 and Land0), then a linear combination is composed on the dependent variables ($v = Bio0 * w$; v is the score and w is weight) and the independent variables ($u = Land0 * t$; u is the score and t is weight). The linear composition of each data set is then built to maximize the covariance between them. This linear composition is called the factor. A second linear composition will be built using the residuals from the first factor and find the linear combinations of both data sets so that their covariance is maximized. The process is repeated by taking residuals from previous factors and producing n-1 factors. For example, if the number of sites (observations) is 89, then 88 factors will be produced. PLS extracts many factors from the data sets. The first factor was explained above.

Values of Euclidean distance from each point to the model in both dependent and independent variables can be plotted. Plots of distances from the two data sets (x's and y's) to the model can help visualization of an outlier, consequently identifying the observation(s) that is(are) located away from the general behavior of the data.

5.2.1 Fitting the Model

PLS produces a number of significant factors using the Cross Validation (CV) method. The process is done by dividing the data into groups (five to nine groups; Wold, 1995). If, for example, the data were divided into five groups, one group (test data) is left out, and the model is fit to other four groups (training data). In SAS there are five CV grouping methods; one, split, block, random, and testset. One, also known as "leave one out", fits the model on n-1 observations and uses the one left out for validation; it is not recommended (Wold, 1995). We used all methods, but found block and split options gave the best results and are similar. An important point to make is that PLS does not require large training and test data sets. The fitted models will be tested using the test data sets, and the predicted values will be compared to that of observed using PRESS (Predictive Residual Sum of Square) to assess the predictive ability of the model; the lower the value, the better the model. However, the model with small PRESS may not be significantly better than a model with fewer factors. The model fit with each number of factors is compared to the best model (based on a randomization of the data).

In SAS, this is done by using options CVTEST (See SAS for PLS statements in Appendix II). SAS gives the root mean PRESS for each model and the significance level of the test of whether that model is different from the one with the lowest PRESS.

5.2.2 Diagnostic Checking and Variable Influence on Projection (VIP)

In each run, and after defining the significant PLS factors (e.g., Tables 4 and 5), we plotted factor's scores, and weights to examine the strength of relationships and irregularities. *Scores* show irregularity grouping and outliers in observations/sites (Figures 5 and 6), whereas, *weights* show irregularity in grouping and outliers for the independent variables. Weights are used in determining VIP (Variable Influence on Projection) that can be used to select the most important variables. VIP is a statistic that PLS produces showing the contribution of the independent variable to the model (Table 5). Sometimes in regression, when the absolute value of the coefficient is small, the contribution of that independent variable to the predicted value is considered small. Consequently that variable is deleted from the model. This may not be the case in PLS using VIP. An independent variable may have a small value of a coefficient but may have a large VIP, implying that this independent variable is important and contributes significantly to the prediction and, therefore, has to be kept in the model. If the value of the VIP and coefficient both are small, that variable may be deleted from the model. Deletion of a variable from a model should also be based on the biological importance or other scientific judgment (see Nash and Bradford, 2001). Wold (1995) indicated a VIP value of less than 0.8 is considered to be small.

Table 4. The significant factors for the preliminary PLS model for the surface water biological properties (5) and landscape metrics (26).

Split-sample Validation for the Number of Extracted Factors			
No. of Extracted Factors	**Root Mean Press**	**t^2**	**$P > t^2$**
0	1.0616	10.7153	0.0440
1	0.9962	0	1.0000
2	1.0925	10.6000	0.0280
3	1.1459	8.05761	0.0780
4	1.1561	11.0342	0.0280
5	1.1535	12.3791	0.0130
6	1.2262	9.6425	0.0370
7	1.2402	9.0255	0.0520
8	1.2315	8.7078	0.0690
9	1.2455	6.7807	0.2090
10	1.2860	6.1018	0.2840
11	1.2498	7.0152	0.1840
12	1.2688	6.4794	0.2350
13	1.2766	6.8627	0.1970
14	1.3077	7.5437	0.1340
15	1.3759	7.5264	0.1240

Minimum root mean PRESS	0.9962
Minimizing number of factors	1
Smallest number of factors with P>0.1	1

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	**Model Effect**		**Dependent Variables**	
	Current	**Total**	**Current**	**Total**
1	29.6554	29.6554	17.6607	17.6607

Table 5. PLS regression coefficient and Variable Influence on Projection (VIP) values for the preliminary model (5 biota and 26 landscape metrics).

Predictor	AgPT	EPT	IBI	Hab	Rich	VIP
Ag_hi	-0.00389	0.00469	-0.00088	0.00280	0.00338	0.11960
Ag_slp_hi	-0.00476	0.00574	-0.00108	0.00342	0.00414	0.13942
Ag_mod	0.05356	-0.06458	0.01211	-0.03852	-0.04658	1.56867
Ag_slp	0.04384	-0.05286	0.00991	-0.03153	-0.03812	1.28396
Ag_slp_mod	0.04479	-0.05401	0.00140	-0.03221	-0.03895	1.31186
Bar_slp_hi	-0.00618	0.00745	-0.00196	0.00444	-0.00537	0.18098
Bar_slp_mod	-0.00866	0.01044	0.01068	0.00623	0.00753	0.25351
Crop_slp	0.04722	-0.05694	0.01140	-0.03396	-0.04106	1.38300
Crop_slp_mod	0.05042	-0.06079	0.00518	-0.03626	-0.04385	1.47666
Past_slp	0.03777	-0.04554	0.00854	-0.02716	-0.03284	1.10608
Pct_bar	-0.00951	0.01146	-0.00215	0.00684	-0.00827	0.27843
Pct_crop	0.03211	-0.03872	0.00726	-0.02310	-0.02793	0.94052
Pct_for	-0.05391	0.06501	-0.01219	0.03877	0.04689	1.57904
Pct_past	0.04648	-0.05605	0.01051	-0.03343	-0.04042	1.36146
Pct_urb	0.02185	-0.02635	-0.00494	-0.01572	-0.01900	0.64000
Pct_wet	-0.00754	0.00909	0.00171	0.00542	0.00656	0.22091
Pct_wtr	0.02541	-0.03064	0.00574	-0.01827	-0.02210	0.74414
PwrPipTl	-0.00544	-0.00656	0.00123	-0.00391	-0.00473	0.15931
Slope3	-0.04075	0.04914	-0.00921	0.02931	0.03544	1.19353
Slp_mod	-0.00465	0.00561	-0.00105	0.00335	0.00405	0.13625
Sd_slp	-0.06240	0.07524	-0.01411	0.04488	0.05426	1.82753
Mean_slp	-0.06168	0.07437	-0.01395	0.04436	0.05363	1.80636
Soil_er	0.03410	-0.04112	0.00771	-0.02453	-0.02966	0.99884
Strmden	-0.03649	0.04400	-0.00825	0.02624	0.03173	1.06877
TotRoad30	0.00822	-0.00991	0.00186	-0.00591	-0.00715	0.24074
TotRoadWS	0.02287	-0.02757	0.00517	-0.01645	-0.01989	0.66980

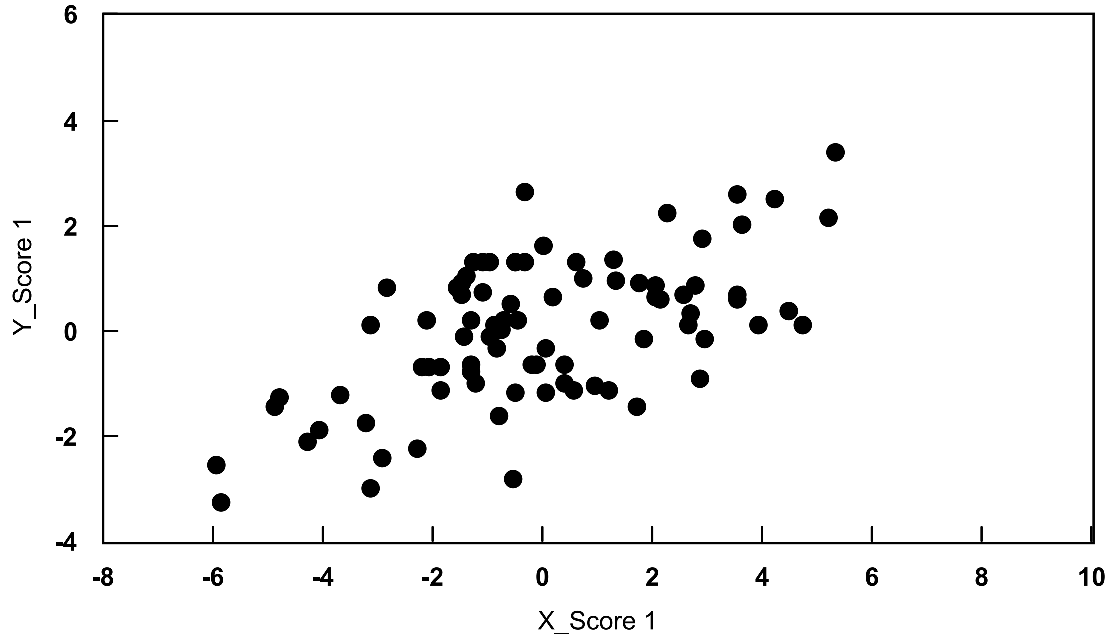

Figure 5. Plot of X- and Y- scores for factor 1 for each of observation from PLS model of biota and 26 landscape metrics.

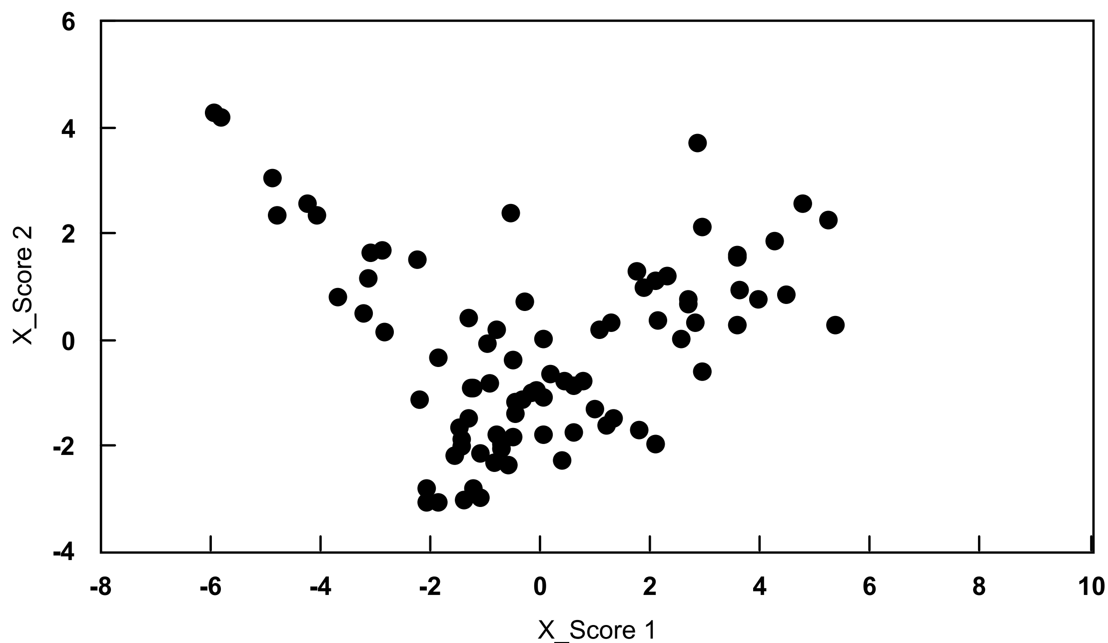

Figure 6. X- scores from the first and second PLS factors for each observation.

5.2.3 PLS Analyses

The way that PLS deals with the number of variables compared to the number of observations, missing observations of some dependent variables, outliers, and collinearity make PLS more applicable than canonical correlation for the Savannah River datasets. For our analyses, therefore, PLS was used for different data sets to fully capture its application to ecological data. Our analysis procedure consisted of the following three steps:

1. We used 26 landscape metrics and the biological metrics (Table 1) including the missing values. The model was reduced to include independent variables (landscape metrics) with VIP >0.8 and a diagnostic check of the model was completed - for finalization.
2. The final model from (1) was analyzed by ecoregion.
3. For ecoregion "Piedmont," missing values were excluded, and the best model found. This model was used to predict for sites that have landscape metrics but no measured biota data.

<u>All Data</u>

The five dependent variables (AgPT, EPT, Hab, Rich, IBI) and the 26 landscape metrics were analyzed. The cross validation was done by dividing the data (n = 86) into two groups; training and testing. The testing data consisted of extracting every 9th value (*Split(9)* option in SAS). Hence, the training data consisted of 78 observations, and the test data consisted of 8 observations. Using training and test data helps in identifying the number of significant factors to include in the final model by cross validation. The model comparison test which compares each model to that with the absolute minimum PRESS, is based on re-randomization of the data (CVTEST option in SAS). The number of significant factors and the percent variation explained by the dependent and independent variables are given in Table 4. Estimates of the PLS regression coefficient and VIP values (Table 5) were examined. The PLS factors accounted for 18 and 30 percent of the variation for the biota and landscape data sets, respectively. Y-score and x-score values for the first factor shows the strength of relationships and the distribution of sites (Figures 5 and 6), and there is no particular clustering pattern in these sites.

The plot of x-weights for the first and second factor indicate which landscape metrics (predictors) are most represented in each factor (Figure 7). It's clear that, for example, the percent of erodible soil, and percent of total area on slope >3 and stream density have high (absolute) weights for both factors while percent forest is most represented in factor 1. Those variables that cluster near the origin (have low weights on both factors) do not contribute much to the predictions capability of the model. Those variables that cluster near each other indicate their equal weight on a factor. The VIP represents the importance of the variable in fitting the PLS for both the dependent and independent variables. The contribution of some of these 26 landscape metrics are low based on the VIP (Ag_hi, Ag_mod, Bar_slp_hi, Bar_slp_mod, Pct_bar, Pct_urb, Pct_wet, Pct_wtr, Slp_mod, Totroad30, and TotroadWS; Table 6). Landscape metrics such as Ag_mod, Ag_slp, Ag_slp_mod have similar VIP values, hence, one may choose one landscape metric to describe agriculture on areas with slope > 3 with moderate erodible soil. The same can be done on Crop_slp, Crop_slp_mod, and Past_slp.

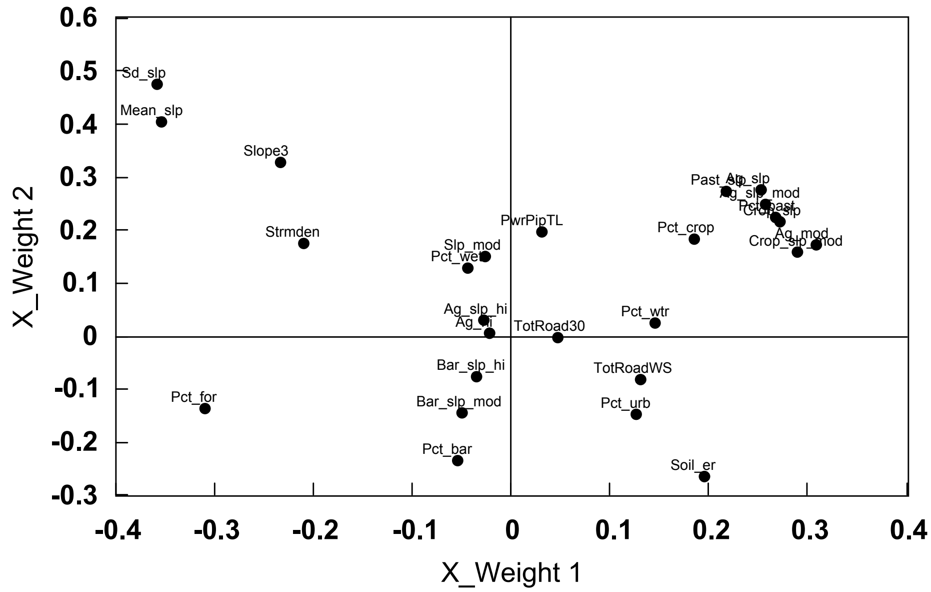

Figure 7. Plot of first and second X- weights for PLS model of biota and 26 landscape metrics.

Table 6. The significant factors of the final PLS model for the 5 biota and 14 landscape metrics and percent variation accounted by PLS factors for the final model.

Blocked Cross Validation for the Number of Extracted Factors			
No. of Extracted Factors	**Root Mean Press**	**t^2**	**$P > t^2$**
0	1.0616	14.7959	0.0040
1	0.9819	11.4172	0.0260
2	0.9686	6.6464	0.2590
3	0.9561	0	1.0000
4	0.9780	8.85220	0.0710
5	0.9769	7.5310	0.1780
6	0.9828	10.2828	0.0550
7	0.9813	6.3798	0.2890
8	0.9845	6.0714	0.3310
9	0.9857	5.1085	0.4540
10	0.9987	5.9617	0.3420
11	1.0221	8.9084	0.1120
12	1.0778	7.6985	0.1590

Minimum root mean PRESS	0.9561
Minimizing number of factors	3
Smallest number of factors with P>0.1	2

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	**Model Effect**		**Dependent Variables**	
	Current	**Total**	**Current**	**Total**
1	51.4485	51.4485	17.4151	17.4151
2	20.9930	72.4414	5.0071	22.4223

The model may perform better if we include only landscape variables with VIP >0.8 (Table 5). Hence, we ran another model that contained only 14 landscape metrics. The final model had two significant factors, which explained 22% and 72% of the variation in the biota (dependent) and landscape metrics (independent), respectively (Table 6). The importance of the 14 landscape metrics were all high (VIP $ 0.8; Table 7), except for Pct_crop (VIP =0.718). X- and Y-scores and weights for the final model are shown in Figures 8 and 9. From Table 7 and Figure 8, a combination of agriculture, crop and pasture were clustered in a group. This group of landscape metrics in addition to slopes, percent of total area with slope > 3%, stream density, percent forest, and erodible soil are contributing the most to the biota in surface water. The contribution of one or many landscape metrics to the surface water biota may be different between ecoregions.

AgPT increased as the agriculture on areas with slope > 3% and erodible soil increased but AgPT decreased as the percent forest, slopes and stream density increased. Pct_crop increases resulted in a decrease in AgPT, further indicating the importance of slope and soil erodibility in relationships to surface water quality.

In the final model, outliers were examined by plotting the x-distance and the y-distance for each site (Appendix I, Figures I-2a and b). There was no evidence of outliers in the data.

Table 7. Coefficient values for the 5 biota and Variable Influence on Projection (VIP) for landscape metrics in the final PLS model.

Predictor	AgPT	EPT	IBI	Hab	Rich	VIP
Ag_mod	0.05972	-0.04367	0.01341	-0.03445	-0.03483	1.08391
Ag_slp	0.05241	0.00084	0.00629	-0.02264	-0.01063	0.99172
Ag_slp_mod	0.05260	-0.00834	0.00759	-0.02450	-0.01534	0.98061
Crop_slp	0.05429	-0.01992	0.00943	-0.02748	-0.02160	0.99831
Crop_slp_mod	0.05602	-0.04027	0.01248	-0.03218	-0.03232	1.01700
Past_slp	0.04606	0.00948	0.00430	-0.01820	-0.00489	0.88841
Pct_for	-0.05861	0.05959	-0.01550	0.03705	0.04270	1.06420
Pct_crop	-0.03837	-0.00408	0.00526	-0.01748	-0.01017	0.71823
Pct_past	0.05386	-0.01863	0.00919	-0.02704	-0.02085	0.99157
Slope3	-0.03418	0.16008	-0.02659	0.04591	0.08870	1.01539
Soil_er	0.02833	-0.12456	0.02090	-0.03647	-0.06938	0.80256
Strmden	-0.03370	0.11052	-0.01959	0.03609	0.06337	0.79064
Sd_slp	-0.05224	0.23189	-0.03885	0.06769	0.12906	1.49047
Mean_slp	-0.05337	0.21200	-0.03621	0.06432	0.11918	1.40765

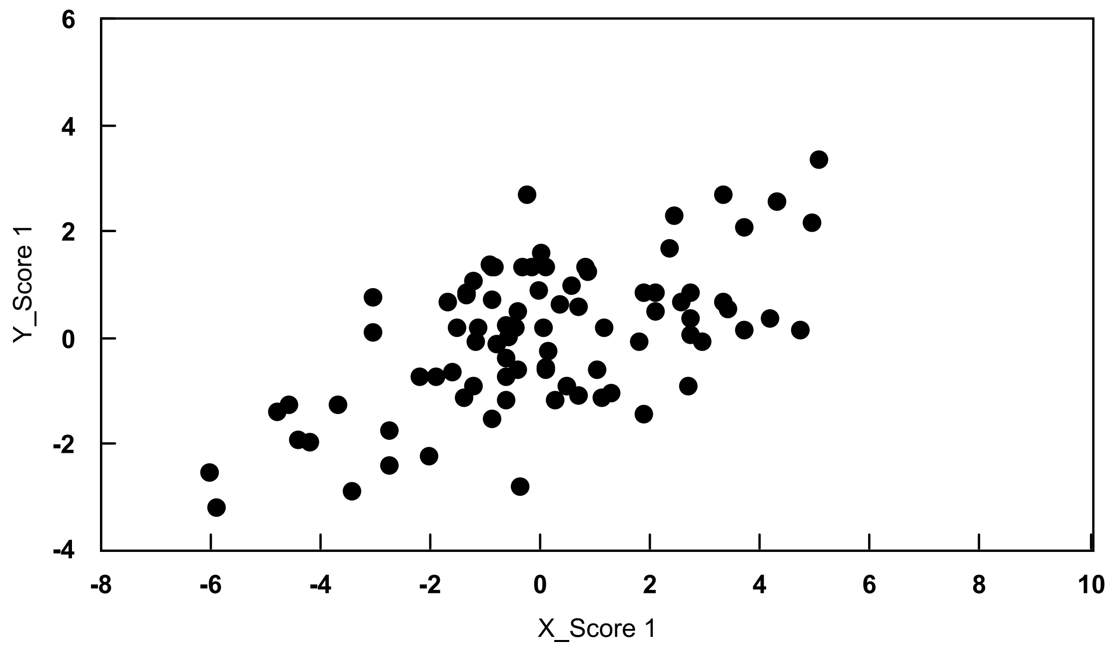

Figure 8. Plot of X- and Y- scores for the first factor of the final PLS model of the 14 landscape metrics and biota.

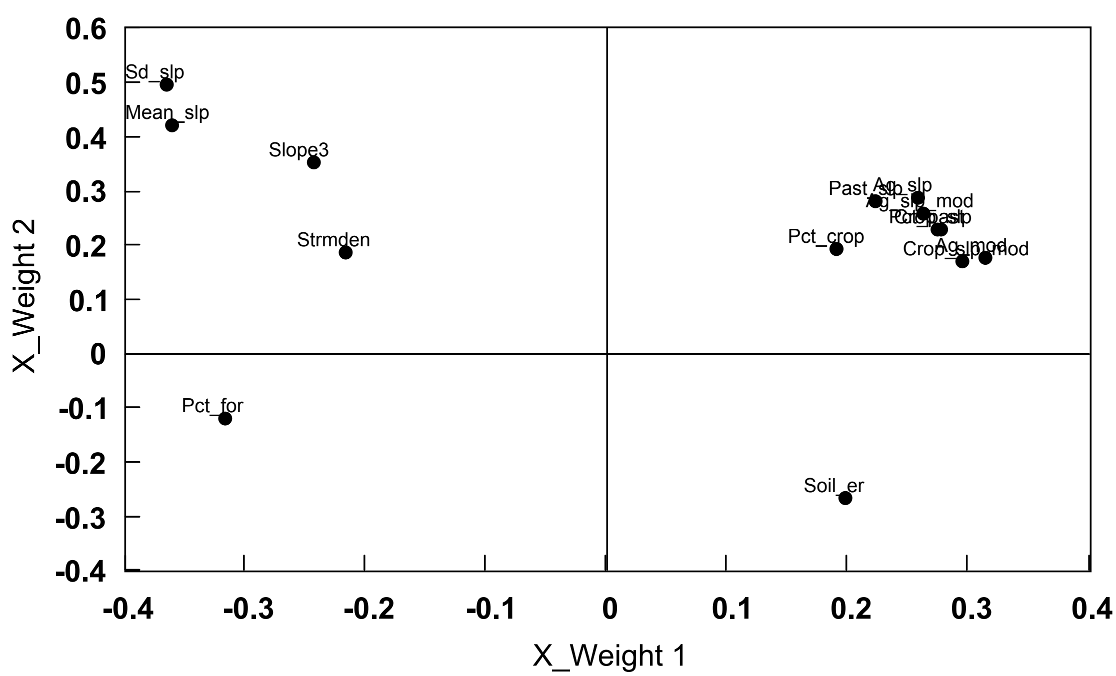

Figure 9. Plot of first and second X- weights for the first PLS final model of biota and the 14 landscape metrics.

By Ecoregion

The final model with the 14 independent variables was rerun by ecoregion.

a) Blue Ridge (BR): There was only one significant factor that accounted for 17% and 74% of the variability for the biota and landscape metrics, respectively (Table 8). Ag_mod, Ag_slp, Ag_slp_mod, Past_slp, percent forest, percent pasture, total area with slope >3%, Sd_slp, Mean_slp (VIP >1) were the most important variables followed by the percent crop and stream density (0.8 < VIP < 1.0). Erodible soil, crop on slopes >3, and crops on areas with slope >3 % and on moderately erodible soil were less important (VIP < 0.8; Table 9). A diagnostic check for the final model indicated a reasonable fit (scores of the first factor; Figure 10). The Blue Ridge ecoregion is characterized by mountainous terrain, predominantly covered in evergreen forest. Barren areas are mainly of two types: transitional areas where the natural forest cover has been removed, and mines. Stream density in the Blue Ridge is the greatest of the three ecoregions comprising the Savannah Basin. Soils are low-to-moderately erodible. Only a small percentage of the total landcover is in agriculture, predominantly pasture, and there are several small urban areas. In total, anthropogenic landcover types account for less than 10% of the land area. The results above indicated that percent forest, forms of agriculture and topography features are the driving elements in effecting surface water quality.

Table 8. The significant factors of the final PLS model for the 5 biota and 14 landscape metrics and percent variation accounted by Partial Least Square factors for the Blue Ridge (BR) ecoregion.

Blocked Cross Validation for the Number of Extracted Factors			
No. of Extracted Factors	**Root Mean Press**	**t^2**	**$P > t^2$**
0	1.3344	11.7283	0.0040
1	1.2758	- 0 -	1.0000
2	1.3442	5.0305	0.4810
3	1.5031	3.1816	0.7950
4	1.5268	6.9633	0.1600
5	1.6327	5.0345	0.4840
6	1.6568	7.0250	0.1700
7	1.8579	7.3210	0.1260
8	2.3660	6.5812	0.2350
9	2.9846	10.8685	0.0050
10	8.7733	10.2386	0.0010
11	1177.274	5.6558	0.2330
12	1177.274	5.6558	0.2330

Minimum root mean PRESS	1.2758
Minimizing number of factors	1
Smallest number of factors with P>0.1	1

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	**Model Effect**		**Dependent Variables**	
	Current	**Total**	**Current**	**Total**
1	73.9938	73.9938	17.4285	17.4285

Table 9. Coefficient and VIP values for biological variables and landscape metrics values for the Blue Ridge (BR) PLS model.

Predictor	AgPT	EPT	IBI	Hab	Rich	VIP
Ag_mod	0.04174	-0.05052	0.01728	-0.01603	-0.03799	1.023
Ag_slp	0.04330	-0.05240	0.01793	-0.01663	-0.03940	1.062
Ag_slp_mod	0.04332	-0.05242	0.01793	-0.01664	-0.03942	1.062
Crop_slp	0.03052	-0.03694	0.01264	-0.01172	-0.02778	0.748
Crop_slp_mod	0.043061	-0.03705	0.01267	-0.01172	-0.02786	0.750
Past_slp	0.04445	-0.05381	0.01841	-0.01708	-0.04046	1.090
Pct_for	-0.04547	0.05504	-0.01883	0.01747	0.04136	1.115
Pct_crop	0.03556	-0.04303	0.01472	-0.01366	-.03236	0.872
Pct_past	0.04115	-0.04981	0.01704	-0.01581	-0.03745	1.009
Slope3	-0.04780	0.05785	-0.01979	0.01836	0.04350	1.172
Soil_er	0.02091	-0.02531	0.00866	-0.00803	-0.01903	0.513
Strmden	-0.03369	0.04078	-0.01395	0.01294	0.03066	0.826
Sd_slp	-0.05318	0.06437	-0.02202	0.02043	0.04840	1.304
Mean_slp	-0.05428	0.06570	-0.02247	0.02085	0.04940	1.331

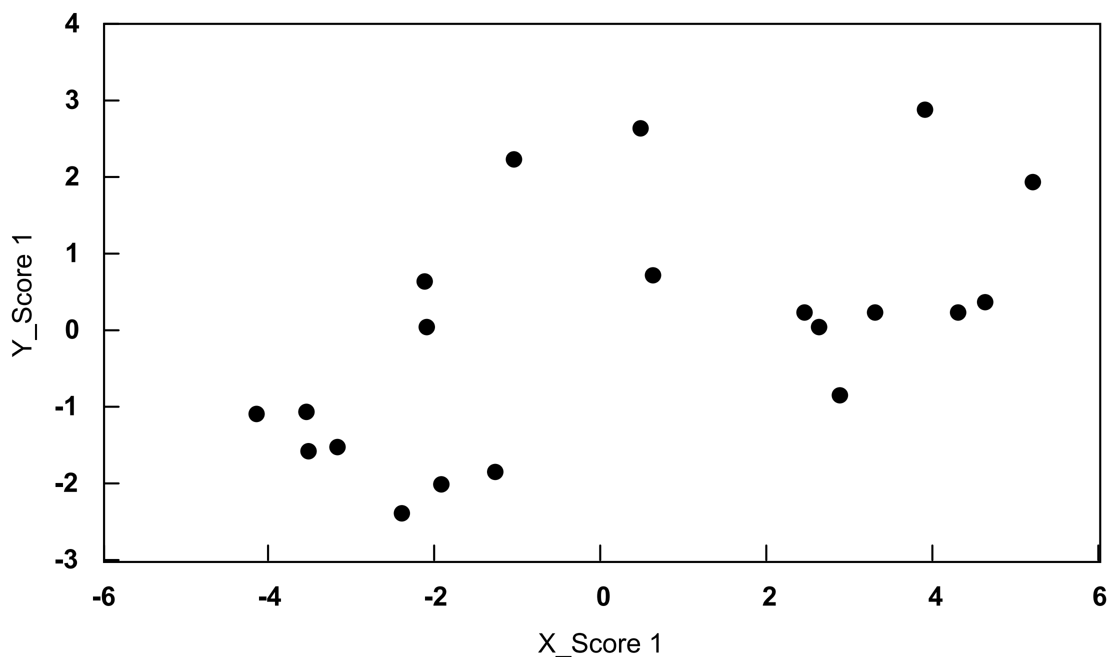

Figure 10. Plot of X- and Y- scores for factor 1 for each of observation from PLS model of 5 biota and 14 landscape variables for Blue Ridge (BR) ecoregion.

b) <u>Piedmont (P)</u>: There were three significant factors that accounted for 29% and 86% of the variability for the biota and landscape metrics, respectively (Table 10). X_ and Y_Scores and weights for the model are shown in Figures 11 and 12. The slopes, percent of the total area on slope >3%, and erodible soil (VIP >1) were the most important variables (Table 11). Percent crop and Crop_slp_mod were also important (VIP . 1). Crop, pasture, and agriculture on the slopes were all grouped in the first quadrant of Figure 12 (0.8 <VIP<1.0); they have positive impact on factor1 and on factor 2. Stream density is less important here than in the Blue Ridge ecoregion. Over half of the Piedmont ecoregion is terrain with slopes greater than 3%. The most predominant landcover is forest, followed by agriculture (with a nearly equal split between pasture and row crops), and transitional barren areas. Agriculture on slopes greater than 3% is evident throughout the ecoregion. All of the highly erodible soils in the basin are in this ecoregion; only very small patches of low-erodible soils occur in the Piedmont, generally along the outer edge of the basin. Percent forest and Stream density is generally less than that of the Blue Ridge, but much greater than that of the Coastal Plains. Agriculture land uses are correlated positively with the biology variable AgPT. AgPT is highly correlated with nutrient concentrations and is representative of a short-time interval; i.e., a high AgPT is likely to indicate a recent influx of nutrients into the water body. Runoff of agricultural fertilizer is a likely source of these nutrients.

Table 10. The significant factors of the final PLS model for the 5 biota and 14 landscape metrics and percent variation accounted by Partial Least Square factors for the Piedmont (P) ecoregion.

Blocked Cross Validation for the Number of Extracted Factors			
No. of Extracted Factors	Root Mean Press	t^2	$P > t^2$
0	1.0645	9.8990	0.0470
1	1.0076	13.0935	0.0130
2	0.9834	9.1652	0.0460
3	0.9761	- 0 -	1.0000
4	0.9988	3.2603	0.7310
5	1.0139	7.1362	0.1760
6	0.9933	5.9928	0.3290
7	1.0201	5.4862	0.3710
8	0.9919	1.9623	0.8760
9	0.9813	2.4957	0.8120
10	0.9878	2.7186	0.7720
11	0.9887	2.5196	0.8070
12	0.9799	2.8799	0.7570

Minimum root mean PRESS	0.9761
Minimizing number of factors	3
Smallest number of factors with P>0.1	3

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	Model Effect		Dependent Variables	
	Current	Total	Current	Total
1	45.7415	45.7415	16.3575	16.3575
2	26.4205	72.1620	8.5655	24.9230
3	13.6948	85.8567	4.2633	29.1863

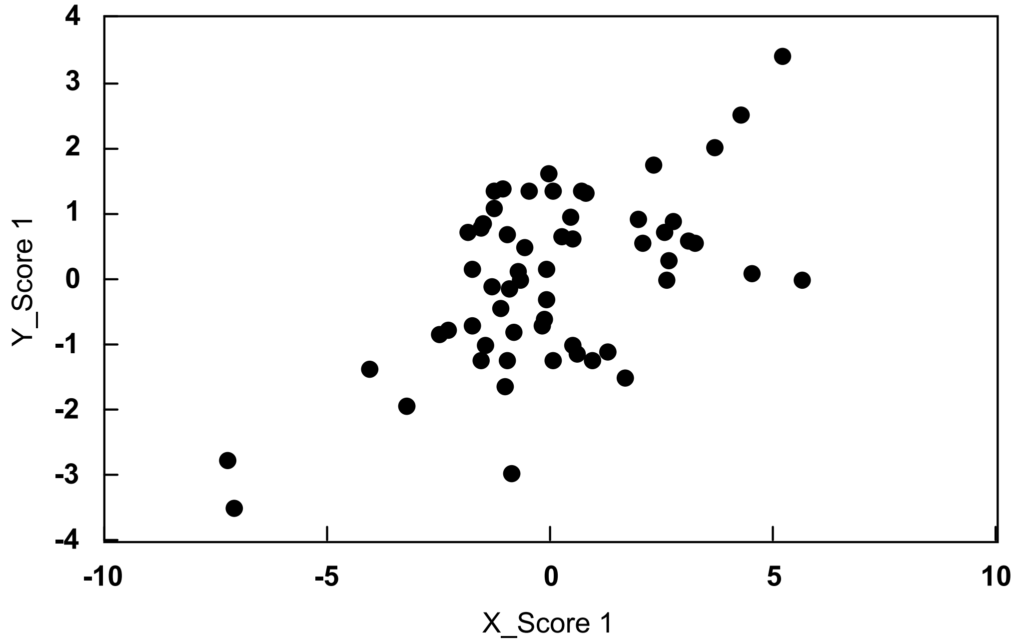

Figure 11. Plot of X- and Y- scores for factor 1 for each of observation from PLS model of 5 biota and 14 landscape variables for Piedmont (P) ecoregion.

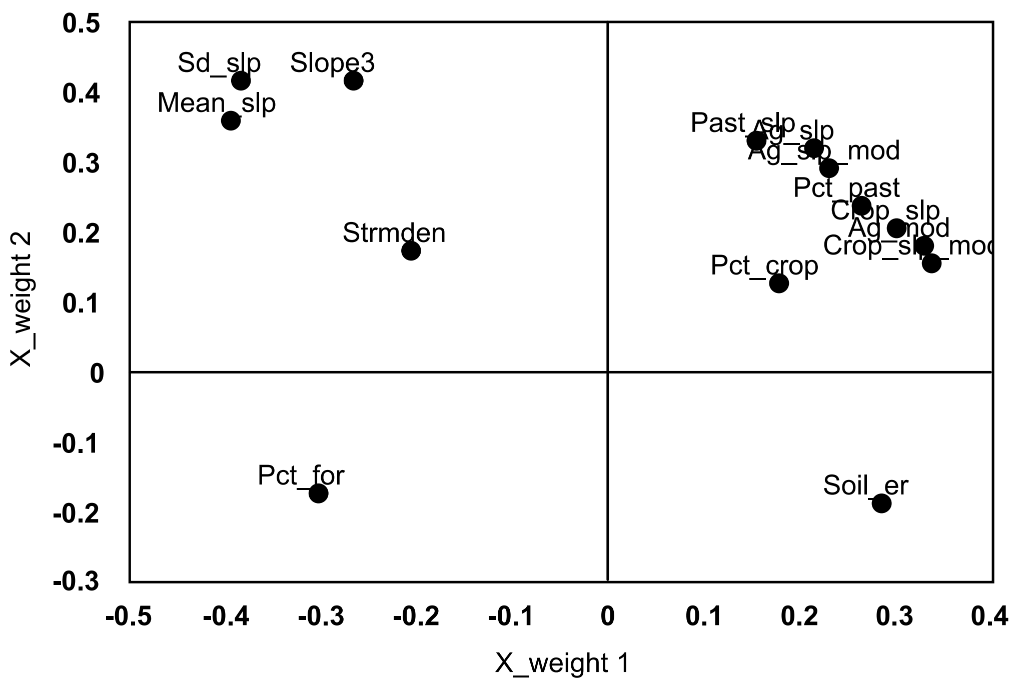

Figure 12. Plot of first and second X- weights for PLS model of biota and the 14 landscape metrics for Piedmont (P) ecoregion.

Table 11. Coefficient and VIP values for the Piedmont (P) PLS model.

Predictor	AgPT	EPT	IBI	Hab	Rich	VIP
Ag_mod	0.06581	-0.05308	0.00437	-0.04933	-0.04007	0.994
Ag_slp	0.05415	0.02204	-0.02300	-0.03363	-0.00086	0.892
Ag_slp_mod	0.0433	0.00505	-0.01496	-0.05919	-0.02000	0.899
Crop_slp	0.06995	-0.03400	-0.00337	-0.03011	-0.02315	0.943
Crop_slp_mod	0.04234	-0.07070	0.01432	-0.09762	-0.07002	1.035
Past_slp	0.04144	0.04328	-0.02874	-0.03137	0.00866	0.815
Pct_for	-0.09665	0.03513	0.00599	-0.02514	-0.00167	0.991
Pct_crop	0.12532	0.00979	-0.02438	0.14737	0.07553	1.084
Pct_past	0.06248	-0.01445	-0.00976	-0.03102	-0.010537	0.885
Slope3	-0.05961	0.18879	-0.06605	-0.03760	0.06369	1.261
Soil_er	-0.04989	-0.17296	0.07080	-0.21475	-0.17145	1.226
Strmden	-0.02735	0.11329	-0.03899	0.02535	0.05918	0.683
Sd_slp	-0.0691	0.24746	-0.09222	0.10386	0.15279	1.373
Mean_slp	-0.0279	0.23252	-0.08418	0.09314	0.14086	1.325

c) Coastal Plain (CP): The scarcity of sampling sites (n=7) in the Coastal Plain precludes an analysis like those completed for the Blue Ridge and Piedmont areas. Therefore, we only presented the percent variation that accounted for two PLS factors in Table 12. X_ and Y_Scores and weights for the model are shown in Figures 13 & 14. However, erodible soil and all agriculture/soil/slope-related landscape metrics yielded VIPs greater than 1 (Table 13; Figure 14). Percent forest, percent crop, and percent pasture were the least important (VIP < 0.8; Table 12) landscape metrics in the Coastal Plain. Soils in this ecoregion are generally of low erodibility, and the terrain is much flatter than the other two ecoregions, hence, area on slope > 3% was not significant as in the Blue Ridge and Piedmont ecoregions. Much of the agriculture is in row crops which are subject to run off, particularly when located on slopes and/or erodible soils. So, while agriculture on slopes and/or moderately erodible soils represent a relatively small percentage of the total landcover, these metrics may cause significant impacts on stream biology on a local scale. Although not significant, all landscape metrics correlated positively with AgPT (Table 13) suggesting, as in the Piedmont, these landscape metrics may be indicative of sources of nutrient inputs to streams.

Table 12. Percent variation accounted by Partial Least Square factors for the Coastal Plain (CP) ecoregion. This model is not significant and therefore it did not have the sample validation for the number of extracted factors.

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	Model Effect		Dependent Variables	
	Current	Total	Current	Total
1	37.8935	37.8935	32.7105	32.7105
2	34.7338	72.6273	24.8842	57.5947

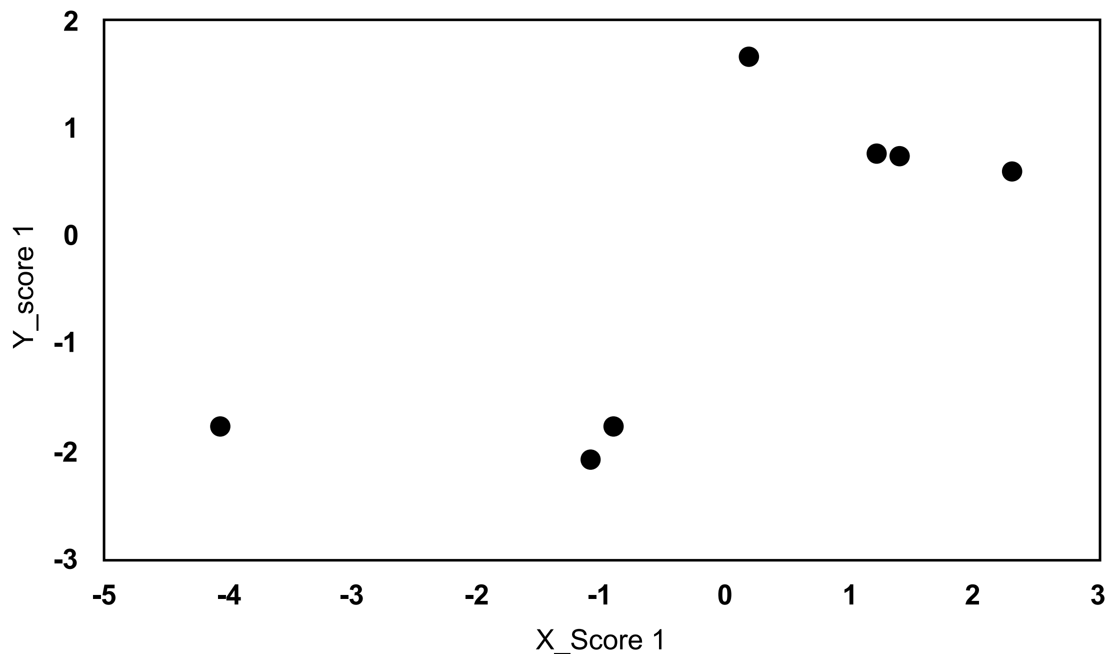

Figure 13. Plot of X- and Y- scores for factor 1 for each of observation from PLS model of 5 biota and 14 landscape variables for Coastal Plain (CP) ecoregion.

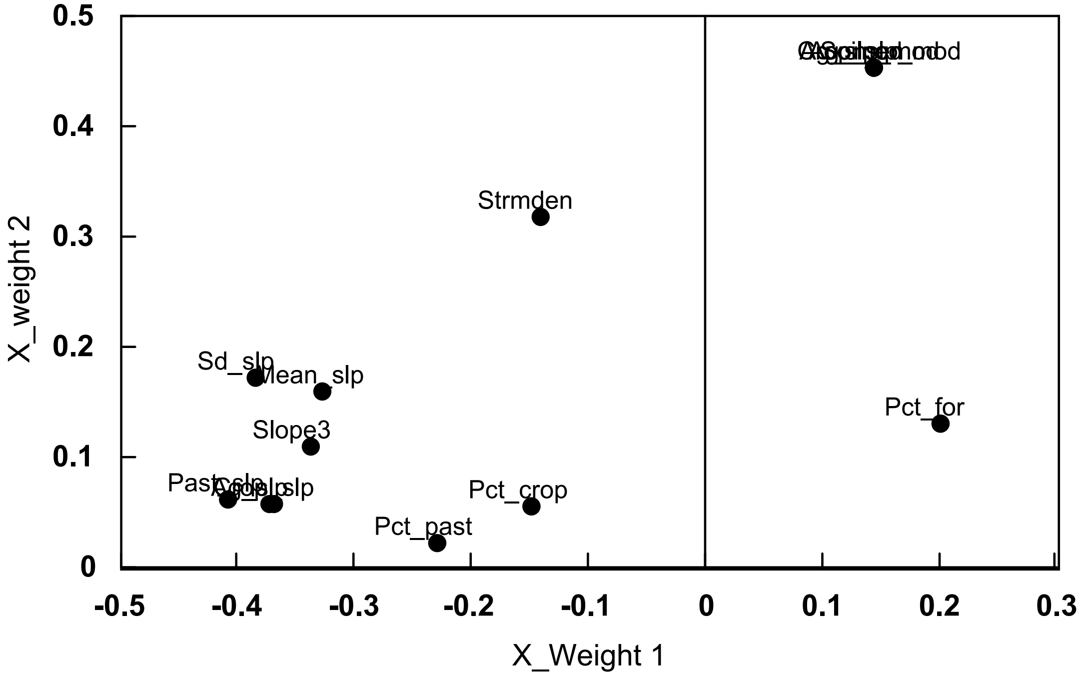

Figure 14. Plot of first and second X- weights for PLS model of biota and the 14 landscape metrics for Coastal Plain (CP) ecoregion.

Table 13. Coefficients of the biota and Variable Influence on Projection (VIP) for landscape metrics for Coastal Plain (CP) ecoregion.

Predictor	AgPT	EPT	Hab	Rich	VIP
Ag_mod	0.200	-0.026	0.085	0.015	1.188
Ag_slp	0.012	0.125	-0.105	0.090	1.064
Ag_slp_mod	0.200	-0.026	0.085	0.015	1.188
Crop_slp	0.012	0.124	-0.104	0.089	1.053
Crop_slp_mod	0.200	-0.026	0.085	0.015	1.188
Past_slp	0.014	0.137	-0.115	0.099	1.164
Pct_for	0.065	-0.059	0.072	-0.031	0.654
Pct_crop	0.018	0.051	-0.039	0.039	0.442
Pct_past	0.001	0.076	-0.066	0.054	0.651
Slope3	0.035	0.116	-0.090	0.088	0.991
Soil_er	0.200	-0.026	0.085	0.015	1.188
Strmden	0.131	0.061	-0.012	0.065	0.882
Sd_slp	0.060	0.134	-0.097	0.104	1.165
Mean_slp	0.056	0.115	-0.082	0.090	1.006

Prediction for the Non-Sampled Sites

Prediction for missing or non sampled measurement(s) for the dependent variables (biota), can be accomplished with PLS. Prediction for missing biota variables are done in two ways; first, the models in "All Data," above, used all missing and non missing biota combined (n=86). As mentioned earlier, PLS has the capability of prediction for the dependent variables if that observation(s) has been measured for the independent variables. The predicted values for the missing biota variables were compared to measured variables by using elevation (as described in Appendix I). Values predicted by the two methods were similar (IBI; $t = 0.25$, $p = 0.80$, $df = 20$; Hab; $t = 0.61$, $p = 0.56$, $df = 8$). Prediction for the missing measurements for the biota using complete data sets that may or may not have missing values in the dependent variables is possible using PLS, an option that is not given by other multivariate analyses.

Another way to predict for dependent variables when they are not measured, is to extract a subset of observations with no missing independent variables and use them in PLS model. This model is then used to predict for observations where the dependent variables (e.g., biota) are not measured. For this, we examined the PLS prediction capability using the data for Piedmont (n=52) with no missing data and 12 landscape metrics (exclude Sd_slp and Mean_slp). The prediction ability of the model was lower when AgPT was included, therefore, the final model did not included AgPT. Another reason for deleting AgPT was that this variable did not have any missing values. The fitted PLS model contains eight significant factors that account for 40% and 99% of the variability in the biota and landscape metrics, respectively (Table 14). In Figure 15, the coefficients for the biota are presented using parallel coordinates superimposed on a bar chart of VIP of the independent variables to enhance an intuitive understanding of the results. The most important landscape metrics in descending order of VIP were percent area on slope >3%, erodible soil, and stream density (Figure 15). Slope more than 3% enhanced EPT and Rich, but decreased IBI and Hab. Erodible soil decreased all four biota. EPT responded positively to Slope3 and negatively to erodible soil other than that it was fairly stable in its relationship to other landscape metrics. Habitat, on the other hand, was positive in stream density, percent forest, percent barren, agriculture on moderately erodible soil, and percent pastures, but responded negatively with other landscape metrics. IBI was enhanced only with the forest. Comparing prediction using the PLS models from Piedmont only and all data gave similar predictions (IBI; $t = 0.54$, $p=0.60$, $df=20$, Hab; $t = 0.26$, $p = 0.81$, $df = 8$). This is probably related to a well sampled study area.

Values may be missing in the dependent and/or independent variables. An observation for which an *independent* variable is missing, will be deleted from the PLS analysis, and no prediction will occur. An observation of which a *dependent* variable is missing but has no missing values for the independent variable, is not included in the analysis, but will have predicted values. To deal with missing values in the dependent variables, PLS requires more than one dependent variable; and the more dependent variables, the better. The recommendation is . 20 dependent variables with missing values of 10!20% (Wold, 1995).

In a study area, access to sampling sites and cost of sampling or other reasons may preclude a complete set of samples on the dependent variables, such as water quality properties. Landscape metrics, on the other hand, (independent variables) can be obtained for all sites. A PLS model that had been developed for a nearby well-sampled area can be used to predict for the missing dependent variables. We developed a PLS model for the "Piedmont" ecoregion using non missing data and without AgPT. The latter model was used to predict for the missing biota (see Appendix III).

Table 14. The significant factors of the final PLS model for the 4 biota and 12 landscape metrics and percent variation accounted by Partial Least Square factors for the Piedmont (P) ecoregion with no missing data.

Blocked Cross Validation for the Number of Extracted Factors			
No. of Extracted Factors	Root Mean Press	t^2	$P > t^2$
0	1.0537	11.2336	0.0140
1	1.0572	14.6516	0.0010
2	1.0117	9.1937	0.0350
3	1.0075	8.1123	0.0650
4	1.0550	12.3995	0.0050
5	1.0221	8.3235	0.0610
6	1.0185	7.6355	0.0890
7	1.0424	10.1230	0.0120
8	1.0350	6.6069	0.1610
9	1.0449	9.3328	0.0160
10	1.0364	2.8318	0.6690
11	0.9873	0	1.000
12	0.9873	3	<0.0001

Minimum root mean PRESS	0.9873
Minimizing number of factors	11
Smallest number of factors with P>0.1	8

Percent Variation Accounted for by PLS Factors				
No. of Extracted Factors	Model Effect		Dependent Variables	
	Current	Total	Current	Total
1	48.9961	48.8861	10.7059	10.7059
2	21.0425	70.0386	14.3107	25.0167
3	10.6343	80.6729	4.0113	29.0280
4	5.4136	86.0862	4.0112	33.0395
5	5.5537	91.6401	2.5154	35.5549
6	4.3696	96.0098	1.4616	37.0165
7	2.0516	98.0614	1.2405	38.2571
8	1.2262	99.2876	1.5392	39.7963

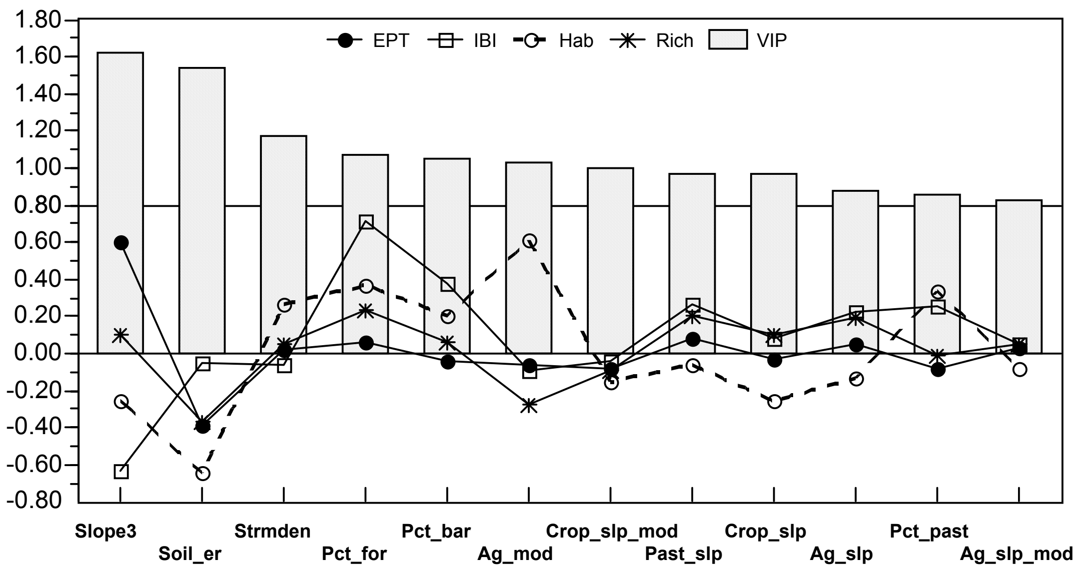

Figure 15. Coefficients of landscape metrics for each biota and the VIP values for PLS model for each landscape metrics with no missing biota for the Piedmont (P) ecoregion. Variability explained for landscape and biota was 99% and 40%, respectively.

5.2.4 Correlation Between the Dependent Variables

All dependent variables (e.g., biota) may be kept in PLS analysis if they are strongly correlated (Wold 1995). But if they are uncorrelated, prediction should be done separately on each of the dependent variables or groups of correlated variables. The strength of collinearity between the dependent variables can be detected by principal component analysis using the percentage of variation that each principal component accounts for. When the number of significant principal components are less than the number of dependent variables, it is an indication of collinearity. Unfortunately, there is no significant test the number of PCA in SAS using PRINCOM. Proc Factor with option (Method = ML; Maximum Likelihood) provide a test of significance for the number of factors. Proc Factor requires multinormality for the dependent variables, and they can be tested using %multinorm macro in SAS (Appendix II). This macro outputs a Q-Q plot of the squared distance and P^2, and if the general behavior is on a straight line, then multicollinearity is implied. The bio data were multinormal using Q-Q plot.

In factor analysis there are two hypotheses that can be tested. First hypothesis (see below) is to test whether there is no correlation between the original variables. The hypothesis (Ho) of independence between the original biota variables is rejected ($p < 0.0001$). The second hypothesis (Ho) states that 2 factors are adequate to describe the biota data ($p = 0.3545 > 0.05$).

	Test	**Df**	P^2	**p >** P^2
Hypothesis(1)	Ho: No common factors Ha: At least one common factor	10	96.5216	<.0001
Hypothesis(2)	Ho: 2 factors are sufficient Ha: More factors are needed	1	0.8571	0.3545

Factor analyses, therefore, indicated that two factors are significant with squared canonical correlations of 0.93 and 0.57 for factors 1 and 2, respectively. Biota variable of EPT and Rich weighted heavily on the first factor. We can also utilize PCA (1) to show the weights of EPT and Rich on the second principal components (Figure 16) and (2) detect any clustering in sites (observations) by plotting the scores of the first two PC (Figure 17). If clustering is evident, then PLS has to be done separately on each cluster of observations. Our data did not show any clustering pattern (Figure 17). From PCA and Factor analyses we concluded that the dependent variables (biota) were correlated and therefore, all were included in PLS.

Collinearity is a big concern in multiple regression (see text above). It is not a concern in PLS. Over fitting the model and consequently having less predictive power of when collinearity exists is still a concern in PLS. PLS overcomes this problem by extracting only the significant factor(s) only as a final model and thereby, preventing over fitting of the model.

In multiple regression, it is important to have enough observations compared to the number of independent variables. The predictive ability is low when the number of independent variables is large in comparison to the number of observations. In contrast, PLS can predict for the dependent variable, even if the above condition exists. PLS has the ability to screen for the most important variables that contribute the most to the variation in the dependent variables. Hence, a final model can be obtained with the most contributing variables to describe a biological phenomena, for example.

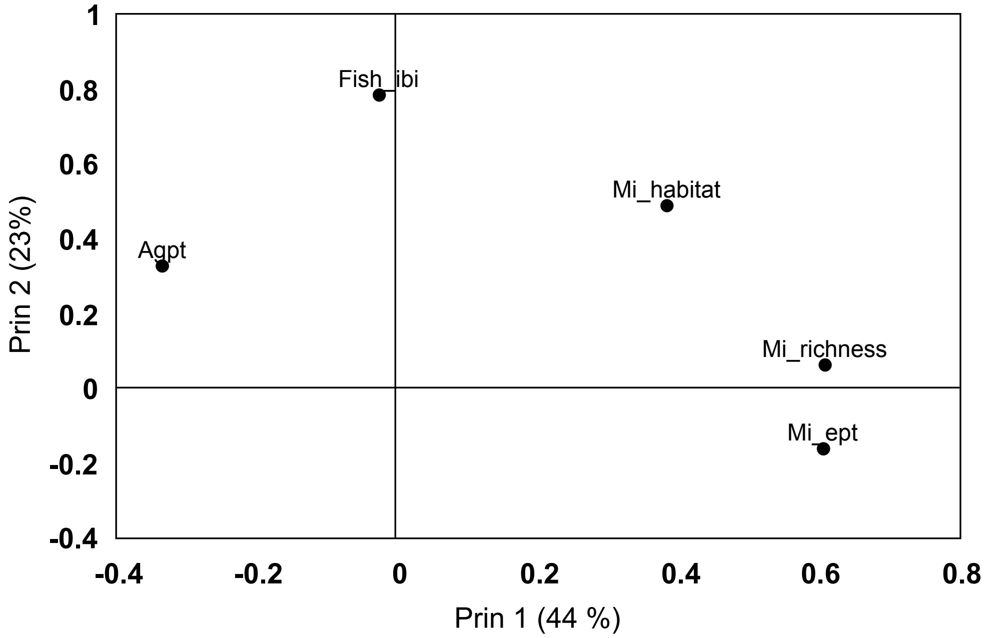

Figure 16. Loading of each of the biota variables (dependent) on the first two principle components (Prin 1 and Prin 2).

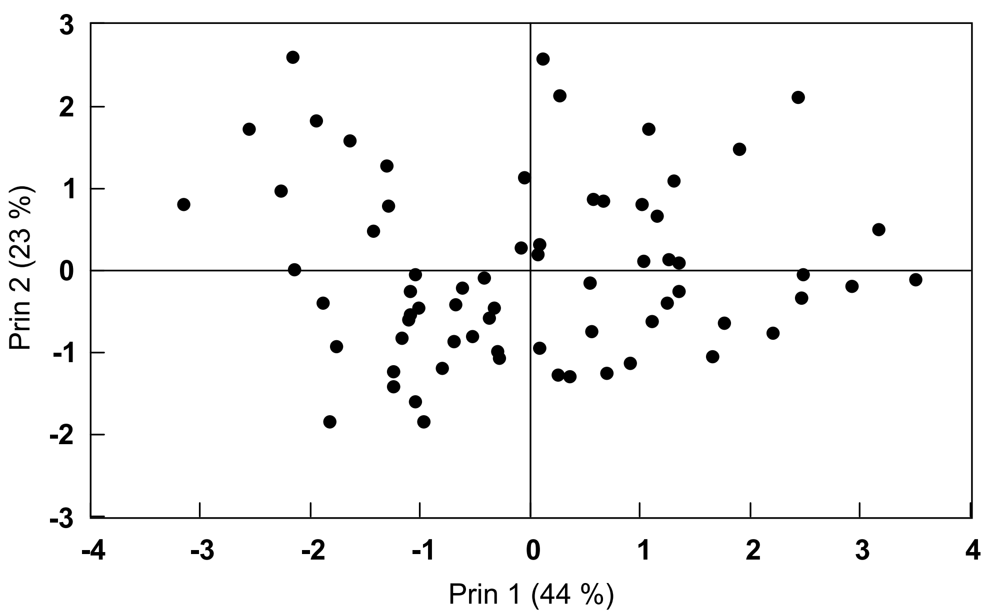

Figure 17. Scores for principle components (Prin 1 versus Prin 2) for the biota variables showing no cluster pattern in sites.

5.2.5 Summary

PLS permitted analyses of the data by ecoregion even on relatively small sample sizes; an option that is not available with other multivariate analyses (e.g., canonical correlation). The analyses revealed that different landscape metrics affect surface water biota based on their spatial association (e.g., ecoregion). Percent forest, percent total area on slope with > 3%, and slopes are the most important landscape variables in Blue Ridge; percent of total area on slope >3%, soil erodibility, percent crop, and Crop_slp_mod were the most important in Piedmont; soil erodibility, Ag_slp_mod and pasture on slopes were the most important landscape variables in Coastal Plain. Stream density was more important in the Blue Ridge than in the Piedmont. Erodible soil was the common landscape variable in Piedmont and Coastal Plain.

When a prediction for missing dependent variables is desired, developing a model from data with no missing measurements for the dependent variables is preferable to PLS including observations with missing dependent variables. Model performance was best for the Piedmont ecoregion, and this model was used to predict the biota from landscape metrics in other locations. Although differences between prediction of Piedmont and all data models were insignificant, still we recommend using the fit model that explains higher variability in the data.

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Section 6
Comparison with Canonical Correlation

Two multivariate analyses, canonical correlation and partial least square (PLS) regression, were conducted to study the relationships between landscape metrics and surface water quality. Canonical correlation is well known in biological and ecological studies, but to our knowledge, this is the first use of PLS to explore relationships in ecological data. Although PLS is unfamiliar to many statisticians, it has been used extensively in chemistry to describe relationships between chemical structures and activity. We wanted to test PLS as a potentially frugal method for landscape ecologists, faced with issues of collinearity and small sample size, to explore relationships which may be used to assess the quality and vulnerability of an ecosystem. We ran PLS only for the biota landscape data to permit detailed exploration of the relationships.

In canonical correlation analyses, collinearity, missing observations, multinormality, and the ratio of number of variables to number of observations are important issues that need to be dealt with prior to analysis. PLS is less subject to these constraints. For the canonical correlation analyses, landscape metrics were selected based on pairwise correlation and discriminant analyses resulting in a total of four landscape metrics. For the PLS analyses, all landscape variables (26) were initially considered in the model and the most important ones were included based on their VIP (> 0.8) in the final model. VIP provides information not only as to how important each landscape variable is (e.g., Table 7) but how similar the contribution is to that of other variables. For example, landscape metrics such as Crop_slp_mod, Ag_slp_mod, and Ag_mod had the same VIP as Soil_er (Table 13) indicating equal contributions of soil erodibility and crop/agriculture on slopes with moderately erodible soil in predicting biota. Therefore, for this group of landscape variables, one variable (e.g., soil erodibility) alone may be used in any model.

Area on slope > 3 has the largest canonical coefficient in the land-biota analysis (page 19) and has the largest VIP in PLS. The only other landscape variable common to canonical correlation and the initial PLS model is Pct_bar which is not greatly important in either method. Pct_bar did not make it to the final model because of its low VIP value. In contrast to the canonical model, the PLS model indicated that landscape metrics such as stream density, percent forest and agriculture on moderately erodible soil were the second important landscape variable group. The other intriguing feature, PLS allowed the analyses by ecoregion which revealed different landscape metrics that relate to surface water biota based on their spatial association (ecoregion, Table 15). It is evident that the importance of any landscape metric is a function of its spatial location in the study area. The importance of percent forest and percent pasture were the highest in Blue Ridge and decreased consistently across the adjacent ecoregions of the study area; the opposite relationship was found for Crop_slp and soil erodibility. Crops on area with slope > 3% with moderately erodible soil, percent crop, percent of areas on slopes > 3% and soil erodibility are the most important landscape variables in the Piedmont. The relative importance of percent pasture on slopes > 3% increased in the Coastal Plain.

Table 15. Rank of the landscape metrics in the PLS model using VIP levels. "All" is the overall model for the three ecoregions.

Metrics	All	Blue Ridge (BR)	Piedmont (P)	Coastal Plain (CP)
Ag_mod	1	1	2	1
Ag_slp	2	1	2	1
Ag_slp_mod	2	1	2	1
Crop_slp	2	3	2	1
Crop_slp_mod	1	3	1	1
Past_slp	2	1	2	1
Pct_for	1	1	2	3
Pct_crop	3	2	1	3
Pct_past	2	1	2	3
Slope3	1	1	1	1
Soil_er	2	3	1	1
Strmden	3	2	3	2
Sd_slp	1	1	1	1
Mean_slp	1	1	1	1

1 = VIP > 1

2 = VIP between 0.8 and 1

3 = VIP < 0.8

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Appendix I

Missing Observations

The total number of sites sampled for water quality parameters was 89, with 18 missing values for at least one metric. When statistical correlation is performed, only sites with complete data are used, so that 71 observations would be included. IBI was the most frequently missing biological metric with 14 sites missing data, 7 of which were in the Coastal Plain (CP). Because of this, CP would be excluded from the analysis and hence, there would be a loss of an ecoregion in spite of the existence of other water biological metrics. Therefore, we substituted for the missing value of a metric its predicted value from a regression model for that metric regressed to a surrogate variable that is not in the canonical correlation analysis. Elevation is a suitable surrogate because it is not included in the canonical correlation analysis and has no missing data.

For each water parameter per ecoregion, we studied its behavior with the elevation to observe the commonality in behavior. When missing value(s) occurred only in a specific ecoregion, a regression model was fit only to that ecoregion data. The IBI had missing data in both the Coastal Plain and Piedmont ecoregions, therefore, we used all non missing values to cover a wider range for better prediction. The model was chosen based on the significant level of the model-F, R^2, and coefficient-t values (p #0.05). When no significant relationship was found, the average value of that water quality parameter in the ecoregion was used to substitute for the missing values. The water quality data for the best fit model is given in Table I-1.

Diagnostic checking of the residuals of the best fit model was performed to test the model assumption concerning the residual for independence and normality (Madansky, 1988).

Assumptions

In relating water biological and landscape metrics, we started with 9 landscape metrics (Strmden, Slope3, Soil_er, Pct_past, Pct_for, Pct_bar, Past_slp, Crop_slp_mod, Ag_mod) and 5 biological variables (AgPT, IBI, Hab, Rich, EPT). For the canonical correlation, we had two matrices: water biological and landscape data sets. Before running the canonical analysis, we tested the data for collinearity, normality, outliers, and the ratio of the number of variables to the number of observations in a sample.

1 - Collinearity: The absolute value of the correlation between variables (within and between each data matrix) was studied (Table I-2). A value of more than 0.9 was considered a sign for collinearity (Griffith and Amrhein, 1997). Therefore, we extracted variables with an absolute value of correlation of less than 0.9. There is a detailed discussion on collinearity in Nash and Bradford (2001).

2 - Normality: Multinormality may not be important when the purpose is only to describe relationships. When testing hypotheses and inferring results, multinormality (joint distribution in canonical) of the variables is important and has to be met (Gittins, 1980). We used a macro (%multinorm; Appendix II) in *SAS* to test for multinormality of the two data sets. The %multinorm produces a chi-square/Q-Q plot which describes the relationships between squared distance and chi-square quantile (Figure I-1). The correlation between squared distance and chi-square can be calculated and used to test the hypothesis of normality (Johnson and Wechern, 2002; Table 4.2, pg 182). For the landscape and biological data sets, the correlation between squared distance and chi-square is 0.9905 (n=84) which is more that of the tabulated (0.9822, n=100,% = 0.01; Table 4.2, Johnson and Wihchern), therefore, not rejecting normality. If the relationship between squared distance and chi-square is linear, it is an indication of a symmetrical distribution without long tails, and multinormality is reached. So, if an observation is found to be an extreme, and it is far from the linear behavior, then that observation has to be studied and deleted if necessary to preserve multinormality.

Table I-1. The best fit model for the water quality variables with their significant levels. Numbers in parentheses are the total number of missing values.

Variable	Ecoregion	Model	P >F	R^2
EC	BR(2)	avg = 90.89		
	P(5)	avg = 92.92[a]		
	CP(1)	avg = 65.85		
pH	BR(2)	7.25 - 0.0005 * elev[b]	0.007	0.37
	P(1)	avg = 6.92		
	CP(1)	avg = 6.05		
DO	BR(6)	avg = 8.33		
	P(3)	avg = 7.13		
	CP(1)	avg = 6.63		
IBI	CP(7) & P(6)	$- 0.02531 * elev + 1.69 * elev^{0.5}$	0.014	0.92
Rich	P(1)	avg = 19.62		
Hab	BR(2)	avg = 75.89		
	P(2)	avg = 75.92		
	CP(1)	77.46 + 0.05 * elev	0.0372	0.70
EPT	P(1)	$157.23 * (elev)^{-0.5}$	<0.0001	0.83

[a] Two values were very high (3260 and 914) and were excluded from the average value.

[b] pH = 6.5 at elevation of 109 m was not in the fitted model (outlier).

Table I-2. Pairwise correlation between all variables in the biota and landscape data.

	Variable	AgPT	EPT	IBI	Hab	Rich	Ag_hi	Ag_slp_hi	Ag_mod	Ag_slp	Ag_slp_mod	Bar_slp_hi	Bar_slp_mod	Crop_slp	Crop_slp_mod	Past_slp
	n=	**86**	**85**	**72**	**81**	**85**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**
1	AgPT	1.000	-0.338	0.109	-0.097	-0.362	0.385	0.393	0.380	0.376	0.309	0.002	-0.120	0.380	0.318	0.316
2	EPT	-0.338	1.000	-0.175	0.197	0.821	-0.080	-0.080	-0.247	-0.205	-0.168	0.006	0.010	-0.281	-0.220	-0.138
3	IBI	0.109	-0.175	1.000	0.150	0.048	0.177	0.181	0.101	0.154	0.146	0.054	0.059	0.077	0.086	0.170
4	Hab	-0.097	0.197	0.150	1.000	0.275	-0.177	-0.175	-0.283	-0.313	-0.329	-0.033	0.000	-0.205	-0.326	-0.314
5	Rich	-0.362	0.821	0.048	0.275	1.000	-0.046	-0.041	-0.207	-0.184	-0.151	0.097	-0.008	-0.292	-0.232	-0.106
1	Ag_hi	0.385	-0.080	0.177	-0.177	-0.046	1.000	0.998	-0.153	-0.037	-0.153	0.428	-0.062	0.006	-0.157	-0.050
2	Ag_slp_hi	0.393	-0.080	0.181	-0.175	-0.041	0.998	1.000	-0.148	-0.031	-0.148	0.426	-0.060	0.012	-0.152	-0.046
3	Ag_mod	0.380	-0.247	0.101	-0.283	-0.207	-0.153	-0.148	1.000	0.879	0.924	-0.175	-0.211	0.651	0.889	0.849
4	Ag_slp	0.376	-0.205	0.154	-0.313	-0.184	-0.037	-0.031	0.879	1.000	0.964	-0.137	-0.241	0.763	0.816	0.955
5	Ag_slp_mod	0.309	-0.168	0.146	-0.329	-0.151	-0.153	-0.148	0.924	0.964	1.000	-0.170	-0.200	0.634	0.862	0.967
6	Bar_slp_hi	0.002	0.006	0.054	-0.033	0.097	0.428	0.426	-0.175	-0.137	-0.170	1.000	-0.072	-0.133	-0.180	-0.118
7	Bar_slp_mod	-0.120	0.010	0.059	0.000	-0.008	-0.062	-0.060	-0.211	-0.241	-0.200	-0.072	1.000	-0.168	-0.100	-0.237
8	Crop_slp	0.380	-0.281	0.077	-0.205	-0.292	0.006	0.012	0.651	0.763	0.634	-0.133	-0.168	1.000	0.765	0.537
9	Crop_slp_mod	0.318	-0.220	0.086	-0.326	-0.232	-0.157	-0.152	0.889	0.816	0.862	-0.180	-0.100	0.765	1.000	0.713
10	Past_slp	0.316	-0.138	0.170	-0.314	-0.106	-0.050	-0.046	0.849	0.955	0.967	-0.118	-0.237	0.537	0.713	1.000
11	Pct_bar	-0.130	-0.127	0.107	0.230	-0.064	0.020	0.023	-0.451	-0.354	-0.448	0.111	0.630	-0.071	-0.386	-0.430
12	Pct_crop	0.269	-0.338	-0.011	0.118	-0.180	-0.026	-0.022	0.243	0.254	0.131	-0.129	-0.223	0.572	0.269	0.068
13	Pct_for	-0.414	0.488	-0.023	0.135	0.339	-0.031	-0.041	-0.586	-0.629	-0.532	0.132	0.161	-0.670	-0.507	-0.513
14	Pct_past	0.411	-0.221	0.161	-0.291	-0.172	0.000	0.006	0.936	0.920	0.928	-0.106	-0.253	0.576	0.746	0.936
15	Pct_urb	0.133	-0.145	-0.263	-0.322	-0.210	0.138	0.143	0.033	0.060	0.056	-0.022	-0.105	0.041	0.066	0.059
16	Pct_wet	-0.088	-0.250	0.023	0.210	-0.100	-0.053	-0.052	-0.220	-0.182	-0.210	-0.057	-0.067	-0.101	-0.217	-0.191
17	Pct_wtr	0.150	-0.194	0.119	-0.061	-0.164	-0.072	-0.069	0.326	0.298	0.285	-0.112	0.114	0.253	0.256	0.272
18	PwrPlpTl	0.059	0.001	-0.069	-0.105	-0.031	-0.023	-0.021	0.148	0.209	0.164	-0.041	-0.105	0.395	0.300	0.091
19	Slope3	-0.138	0.577	-0.125	-0.131	0.268	-0.092	-0.085	0.015	0.126	0.187	-0.054	0.115	-0.070	0.109	0.197
20	Slp_mod	-0.083	0.290	-0.024	-0.286	0.115	-0.270	-0.261	0.297	0.275	0.412	-0.300	0.197	-0.015	0.370	0.366
21	Sd_slp	-0.178	0.697	-0.085	0.192	0.445	-0.101	-0.096	-0.177	-0.151	-0.100	-0.097	-0.028	-0.252	-0.144	-0.081
22	Mean_slp	-0.210	0.676	-0.108	0.191	0.417	-0.090	-0.086	-0.221	-0.193	-0.136	-0.083	-0.006	-0.301	-0.181	-0.114
23	Soit_er	0.098	0.005	0.143	-0.459	-0.035	0.100	0.097	0.431	0.276	0.414	0.101	0.170	-0.004	0.438	0.362
24	Strmden	-0.263	0.292	-0.219	0.124	0.190	-0.127	-0.120	-0.079	-0.074	-0.018	-0.131	0.171	-0.111	0.014	-0.046
25	TotRoad30	0.068	-0.142	-0.081	-0.077	-0.146	-0.008	-0.004	0.124	0.144	0.145	-0.086	-0.193	0.147	0.171	0.121
26	TotRoadWS	0.146	-0.137	-0.203	-0.341	-0.176	0.076	0.084	0.135	0.226	0.193	-0.098	-0.193	0.148	0.106	0.227

Table I-2 *(Continued)*

	Variable	Pct_bar	Pct_crop	Pct_for	Pct_past	Pct_urb	Pct_wet	Pct_wtr	PwrPlpTL	Slope3	Slp_mod	Sd_slp	Mean_slp	Soil_er	Strmden	TotRoad30	TotRoadWS
	n=	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**	**86**
1	AgPT	-0.130	0.269	-0.414	0.411	0.133	-0.088	0.150	0.059	-0.138	-0.083	-0.178	-0.210	0.098	-0.263	0.068	0.146
2	EPT	-0.127	-0.338	0.488	-0.221	-0.145	-0.250	-0.194	0.001	0.577	0.290	0.697	0.676	0.005	0.292	-0.142	-0.137
3	IBI	0.107	-0.011	-0.023	0.161	-0.263	0.023	0.119	-0.069	-0.125	-0.024	-0.085	-0.108	0.143	-0.219	-0.081	-0.203
4	Hab	0.230	0.118	0.135	-0.291	-0.322	0.210	-0.061	-0.105	-0.131	-0.286	0.192	0.191	-0.459	0.124	-0.077	-0.341
5	Rich	-0.064	-0.180	0.339	-0.172	-0.210	-0.100	-0.164	-0.031	0.268	0.115	0.445	0.417	-0.035	0.190	-0.146	-0.176
1	Ag_hi	0.020	-0.026	-0.031	0.000	0.138	-0.053	-0.072	-0.023	-0.092	-0.270	-0.101	-0.090	0.100	-0.127	-0.008	0.076
2	Ag_slp_hi	0.023	-0.022	-0.041	0.006	0.143	-0.052	-0.069	-0.021	-0.085	-0.261	-0.096	-0.086	0.097	-0.120	-0.004	0.084
3	Ag_mod	-0.451	0.243	-0.586	0.936	0.033	-0.220	0.326	0.148	0.015	0.297	-0.177	-0.221	0.431	-0.079	0.124	0.135
4	Ag_slp	-0.354	0.254	-0.629	0.920	0.660	-0.182	0.298	0.209	0.126	0.275	-0.151	-0.193	0.276	-0.074	0.144	0.226
5	Ag_slp_mod	-0.448	0.131	-0.532	0.928	0.056	-0.210	0.285	0.164	0.187	0.412	-0.100	-0.136	0.414	-0.018	0.145	0.193
6	Bar_slp_hi	0.111	-0.129	0.132	-0.106	-0.022	-0.057	-0.112	-0.041	-0.054	-0.300	-0.097	-0.083	0.101	-0.131	-0.086	-0.098
7	Bar_slp_mod	0.630	-0.223	0.161	-0.253	-0.105	-0.067	0.114	-0.105	0.115	0.197	-0.028	-0.006	0.170	0.171	-0.193	-0.193
8	Crop_slp	-0.071	0.572	-0.670	0.576	0.041	-0.101	0.253	0.395	-0.070	-0.015	-0.252	-0.301	-0.004	-0.111	0.147	0.148
9	Crop_slp_mod	-0.386	0.269	-0.507	0.746	0.066	-0.217	0.256	0.300	0.109	0.370	-0.144	-0.181	0.438	0.014	0.171	0.106
10	Past_slp	-0.430	0.068	-0.513	0.936	0.059	-0.191	0.272	0.091	0.197	0.366	-0.081	-0.114	0.362	-0.046	0.121	0.227
11	Pct_bar	1.000	0.052	0.009	-0.439	-0.159	0.094	0.004	-0.060	-0.252	-0.355	-0.235	-0.226	-0.353	-0.068	-0.151	-0.158
12	Pct_crop	0.052	1.000	-0.735	0.175	-0.063	0.328	0.114	0.228	-0.598	-0.474	-0.416	-0.444	-0.493	-0.296	0.056	0.031
13	Pct_for	0.009	-0.735	1.000	-0.608	-0.319	-0.294	-0.244	-0.199	0.472	0.253	0.492	0.529	0.209	0.323	-0.331	-0.393
14	Pct_past	-0.439	0.175	-0.608	1.000	0.047	-0.187	0.314	0.106	0.044	0.255	-0.162	-0.203	0.369	-0.101	0.118	0.200
15	Pct_urb	-0.159	-0.063	-0.319	0.047	1.000	-0.068	-0.117	0.056	0.049	0.080	-0.061	-0.071	0.116	-0.096	0.541	0.812
16	Pct_wet	0.094	0.328	-0.294	-0.187	-0.068	1.000	0.013	0.003	-0.499	-0.383	-0.267	-0.238	-0.530	-0.154	0.250	-0.089
17	Pct_wtr	0.004	0.114	-0.244	0.314	-0.117	0.013	1.000	-0.107	-0.007	0.091	-0.093	-0.111	0.094	-0.015	-0.104	-0.181
18	PwrPlpTl	-0.060	0.228	-0.199	0.106	0.056	0.003	-0.107	1.000	-0.022	-0.004	-0.053	-0.073	-0.039	0.050	0.203	0.028
19	Slope3	-0.252	-0.598	0.472	0.044	0.049	-0.499	-0.007	-0.022	1.000	0.740	0.764	0.753	0.376	0.487	-0.062	0.026
20	Slp_mod	-0.355	-0.474	0.253	0.255	0.080	-0.383	0.091	-0.004	0.740	1.000	0.368	0.340	0.705	0.462	-0.042	0.074
21	Sd_slp	-0.235	-0.416	0.492	-0.162	-0.061	-0.267	-0.093	-0.053	0.764	0.368	1.000	0.979	-0.013	0.353	0.044	-0.124
22	Mean_slp	-0.226	-0.444	0.529	-0.203	-0.071	-0.238	-0.111	-0.073	0.753	0.340	0.979	1.000	-0.028	0.366	0.054	-0.150
23	Soil_er	-0.353	-0.493	0.209	0.369	0.116	-0.530	0.094	-0.039	0.376	0.705	-0.013	-0.028	1.000	0.201	-0.079	0.044
24	Strmden	-0.068	-0.296	0.323	-0.101	-0.096	-0.154	-0.015	0.050	0.487	0.462	0.353	0.366	0.201	1.000	-0.020	-0.091
25	TotRoad30	-0.151	0.056	-0.331	0.118	0.541	0.250	-0.104	0.203	-0.062	-0.042	0.044	0.054	-0.079	-0.020	1.000	0.566
26	TotRoadWS	-0.158	0.031	-0.393	0.200	0.812	-0.089	-0.181	0.028	0.026	0.074	-0.124	-0.150	0.044	-0.091	0.566	1.000

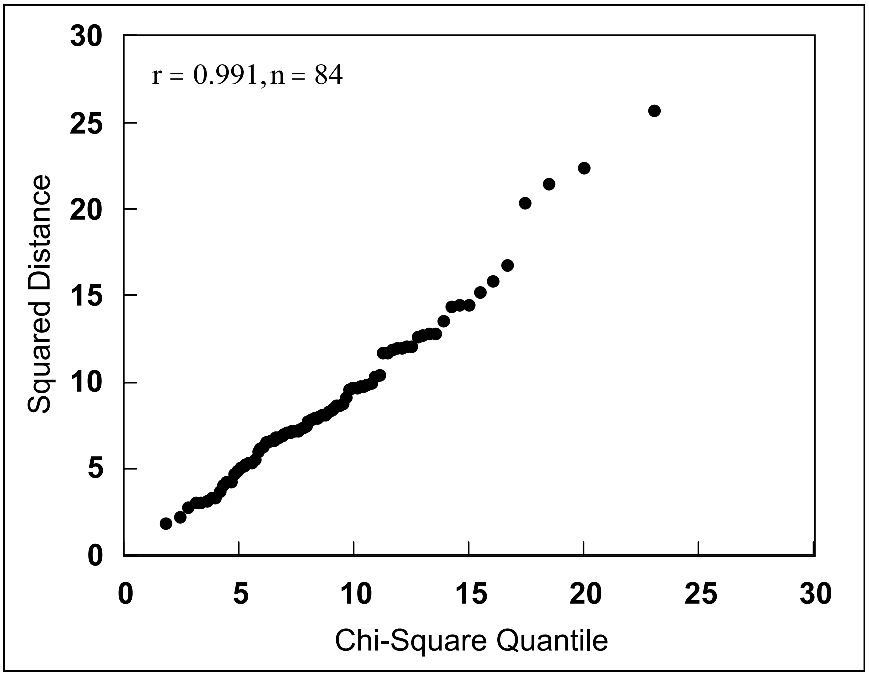

Figure I-1. The squared distance and chi-square quantile for the landscape and biota variables used for the canonical correlation analyses.

3 - Number of Variables to Number of Observations Ratio: The number of variables in each data set has to be equal to or more than two (Thorndike, 1978). When the number of variables becomes large with respect to the number of observations, then it is necessary to consider the ratio of the number of variables to the number of observations in a sample (Gitten, 1980). A high ratio will introduce a bias in estimating the canonical correlation coefficient and roots (eigenvalues). Therefore, the results will not be reliable. A range for the variable to sample ratio of 0.025-0.05 as minimum has been recommended (Barcikowski & Stevens, 1975; Thorndike, 1978). A detailed discussion of the variable/sample ratio is given in Gitten (1980, Chap. 13). One way to reduce the number of variables is to choose the ones that contribute the most to the variability of the data. We therefore, we perform a discriminate analyses with stepwise selection using STEPDISC Procedure (Proc StepDisc; SAS) for each of the biological and landscape data sets by ecoregion to reduce the number of variables. SAS statements below describe the discriminate procedure.

```
Proc stepdisc data=wl all sle=0.35 sls=0.15;
    class Ecoregion;
    var agpt ibi rich hab ept ;
*     var Pct_crop Crop_slp Pct_past Past_slp Pct_wet Pct_bar Pct_wtr
          sd_slp Slope3 Mean_slp TotRoad30 TotRoadWS strmden soil_er;
run;
```

Results for biological parameters were AgPT, IBI, Hab, Rich, and EPT and for landscape variables were Past_slp, Pct_bar, Soil_er and Slope3. The ratio of the number of variables to samples were 0.107 (9/84). Being aware of the external information about the variables and the study area, we believed this ratio was satisfactory. The ratio of the number of variables to samples for chemical/landscape metrics and chemical/biological metrics was 0.09 (7/77) and 0.009 (7/77), respectively.

Outliers were examined by plotting the x-distance and the y-distance for each site (Appendix I, Figures I-2a and b). There was no evidence of outliers in the data.

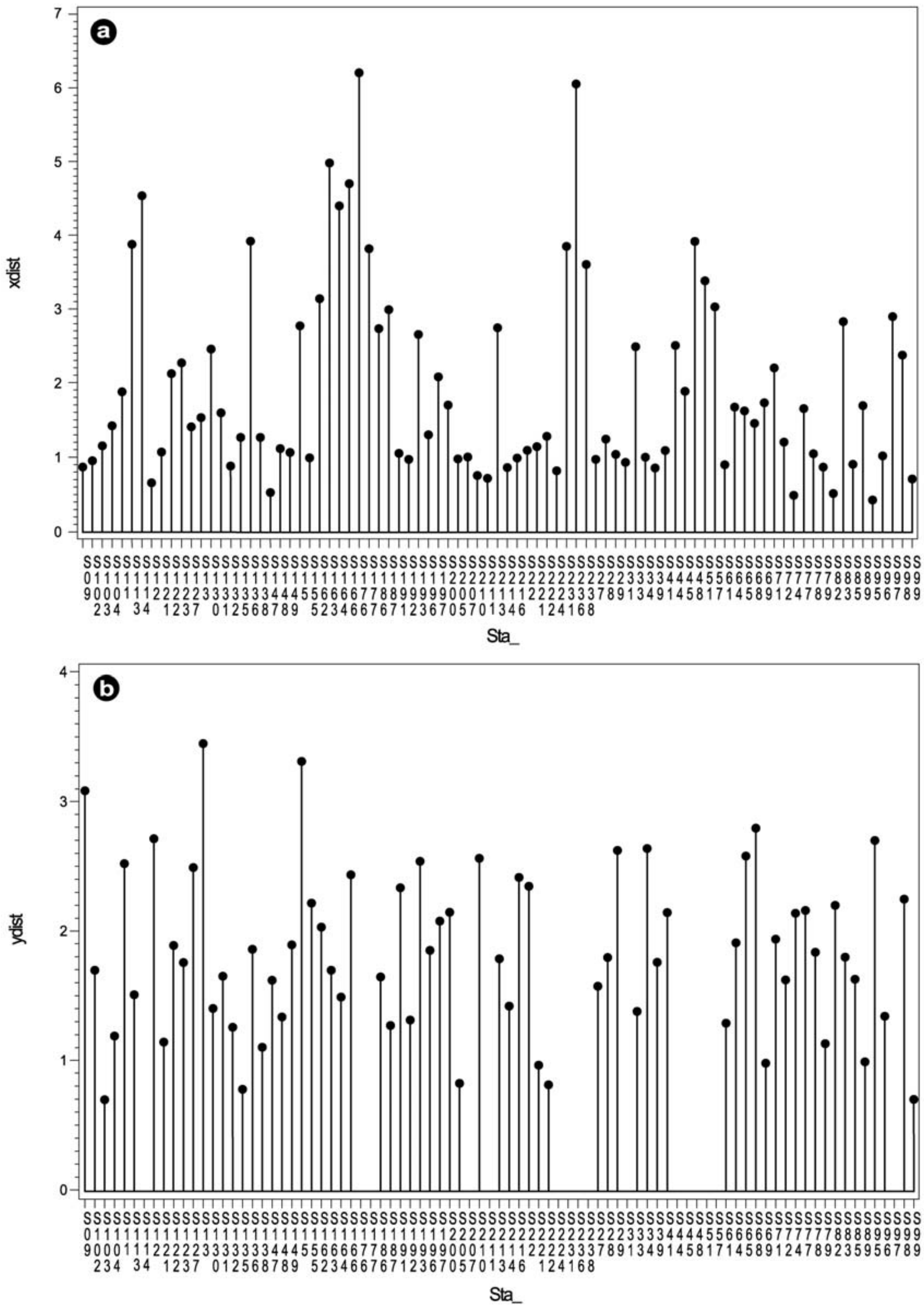

Figure I-2. (a) x-distance and (b) y-distance to the model.

Appendix II
%multinorm SAS Macro

```
**** From SAS ************************************************************;
****  Multinormality test, Principle components and factor analyses  ******;
%multnorm(data=Chloud.watland,var=AgPT EPT IBI Hab Rich)
*************************************************************************/

%macro multnorm (
       data=_last_ ,    /*            input data set           */
       var=        ,    /* REQUIRED:  variables for test       */
                        /* May NOT be a list e.g. var1-var10   */
       plot=yes    ,    /*           create chi-square plot?  */
       hires=yes        /*            high resolution plot?    */
              );
options nonotes;
%let lastds=&syslast;

/* Verify that VAR= option is specified */
%if &var= %then %do;
    %put ERROR: Specify test variables in the VAR= argument;
    %goto exit;
%end;

/* Parse VAR= list */
%let _i=1;
%do %while (%scan(&var,&_i) ne %str() );
   %let arg&_i=%scan(&var,&_i);
   %let _i=%eval(&_i+1);
%end;
%let nvar=%eval(&_i-1);

/* Remove observations with missing values */
%put NOTE: Removing observations with missing values...;
data _nomiss;
  set &data;
  if nmiss(of &var )=0;
  run;

/* Quit if covariance matrix is singular */
%let singular=nonsingular;
%put NOTE: Checking for singularity of covariance matrix...;
```

```
proc princomp data=_nomiss outstat=_evals noprint;
  var &var ;
  run;
%if &syserr=3000 %then %do;
  %put NOTE: PROC PRINCOMP required for singularity check.;
  %put NOTE: Covariance matrix not checked for singularity.;
  %goto findproc;
%end;
data _null_;
  set _evals;
  where _TYPE_='EIGENVAL';
  if round(min(of &var ),1e-8)<=0 then do;
    put 'ERROR: Covariance matrix is singular.';
    call symput('singular','singular');
  end;
  run;
%if &singular=singular %then %goto exit;

%findproc:
/* Is IML or MODEL available for analysis? */
%let mult=yes; %let multtext=%str( and Multivariate);
%put NOTE: Checking for necessary procedures...;
proc iml; quit;
%if &syserr=0 %then %goto iml;
proc model; quit;
%if &syserr=0 and
    (%substr(&sysvlong,1,9)>=6.09.0450 and %substr(&sysvlong,3,2) ne 10)
    %then %goto model;
%put NOTE: SAS/ETS PROC MODEL with NORMAL option or SAS/IML is required;
%put %str(     ) to perform tests of multivariate normality.  Univariate;
%put %str(     ) normality tests will be done.;
%let mult=no; %let multtext=;
%goto univar;

%iml:
proc iml;
  reset;
  use _nomiss;  read all var {&var} into _x;   /* input data */
  /* compute mahalanobis distances */
  _n=nrow(_x); _p=ncol(_x);
  _c=_x-j(_n,1)*_x[:,];         /* centered variables    */
  _s=(_c`*_c)/_n;              /* covariance matrix     */  _rij=_c*inv(_s)*_c`;          /* mahalanobis angles
*/

  /* get values for probability plot and output to data set */
  %if &plot=yes %then %do;
  _d=vecdiag(_rij#(_n-1)/_n);   /* squared mahalanobis distances */
  _rank=ranktie(_d);            /* ranks of distances    */
  _chi=cinv((_rank-.5)/_n,_p);  /* chi-square quantiles  */
  _chiplot=_d||_chi;
```

```
  create _chiplot from _chiplot [colname={'MAHDIST' 'CHISQ'}];
  append from _chiplot;
  %end;

  /* Mardia tests based on multivariate skewness and kurtosis */
  _b1p=(_rij##3)[+,+]/(_n##2);                  /* skewness */
  _b2p=trace(_rij##2)/_n;                       /* kurtosis */
  _k=(_p+1)#(_n+1)#(_n+3)/(_n#((_n+1)#(_p+1)-6)); /* small sample correction */
  _b1pchi=_b1p#_n#_k/6;                         /* skewness test statistic */
  _b1pdf=_p#(_p+1)#(_p+2)/6;                    /*   and df              */
  _b2pnorm=(_b2p-_p#(_p+2))/sqrt(8#_p#(_p+2)/_n); /* kurtosis test statistic */
  _probb1p=1-probchi(_b1pchi,_b1pdf);           /* skewness p-value */
  _probb2p=2*(1-probnorm(abs(_b2pnorm)));       /* kurtosis p-value */

  /* output results to data sets */
  _names={"Mardia Skewness","Mardia Kurtosis"};
  create _names from _names [colname='TEST'];
  append from _names;
  _probs=(_n||_b1p||_b1pchi||_probb1p) // (_n||_b2p||_b2pnorm||_probb2p);
  create _values from _probs [colname={'N' 'VALUE' 'STAT' 'PROB'}];
  append from _probs;
quit;

data _mult;
  merge _names _values;
  run;

%univar:
/* get univariate test results */
proc univariate data=_nomiss noprint;
  var &var;
  output out=_stat normal=&var ;
  output out=_prob  probn=&var ;
  output out=_n         n=&var ;
  run;

data _univ;
  set _stat _prob _n;
  run;

proc transpose data=_univ name=variable
             out=_tuniv(rename=(col1=stat col2=prob col3=n));
   var &var ;
   run;
data _both;
  length test $15.;
  set _tuniv
     %if &mult=yes %then _mult;;
  if test='' then if n<=2000 then test='Shapiro-Wilk';
                else test='Kolmogorov';
```

```
  run;
proc print data=_both noobs split='/';
  var variable n test      %if &mult=yes %then value;
      stat prob;
  title "Univariate&multtext Normality Tests";
  label variable="Variable"
          test="Test"       %if &mult=yes %then
         value="Multivariate/Skewness &/Kurtosis";
          stat="Test/Statistic/Value"
          prob="p-value";
  run;
%if &plot=yes %then
   %if &mult=yes %then %goto plotstep;
   %else %goto plot;
%else %goto exit;

%model:
/* Multivariate and Univariate tests with MODEL */
proc model data=_nomiss;
  %do _i=1 %to &nvar;
    &&arg&_i = _a;
  %end;
  fit &var / normal;
  title "Univariate&multtext Normality Tests";
  run;
%if &plot ne yes %then %goto exit;

%plot:
/* compute values for chi-square Q-Q plot */
proc princomp data=_nomiss std out=_chiplot noprint;
  var &var ;
  run;
%if &syserr=3000 %then %do;
  %put ERROR: PROC PRINCOMP in SAS/STAT needed to do plot.;
  %goto exit;
%end;
data _chiplot;
  set _chiplot;
  mahdist=uss(of prin1-prin&nvar );
  keep mahdist;
  run;
proc rank data=_chiplot out=_chiplot;
  var mahdist;
  ranks rdist;
  run;
data _chiplot;
  set _chiplot nobs=_n;
  chisq=cinv((rdist-.5)/_n,&nvar);
  keep mahdist chisq;
  run;
```

```
%plotstep:
/* Create a chi-square Q-Q plot
   NOTE: Very large sample size is required for chi-square asymptotics
   unless the number of variables is very small.
*/
%if &hires=yes %then proc gplot data=_chiplot;
%else                proc  plot data=_chiplot;;
   plot mahdist*chisq;
   label mahdist="Squared Distance"
         chisq="Chi-square quantile";
   title "Chi-square Q-Q plot";
   run;
   quit;
%if &syserr=3000 %then %do;
   %put NOTE: PROC PLOT will be used instead.;
   %let hires=no;
   %goto plotstep;
%end;
%exit:
options notes _last_=&lastds;
title;
%mend;
Proc corr data=_chiplot;
    var mahdist chisq;
   run;
   quit;
Proc sort data=_chiplot out=a;
    by mahdist chisq;
   run;
   quit;
Proc plot data = a vpercent=17 hpercent=90;
    plot mahdist*chisq / vref = 12.5 href=13;;
run;
options ps=255 ls=80;
Proc print data = a;/* export this data to other graphing software for better presentation*/
    var mahdist chisq;
run;
```

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Appendix III

SAS Statements for PLS and Diagnostic Checking

```
libname Factor 'c:\abc' ;

** Principle component to examine srength of Colinearity in the dependent variables ******** ;
Proc Princomp data=chloud.watland out=prin;
    var AgPT EPT IBI Hab Rich;
run;



** Factor analyses to find the numner of significant factor for the biota  ******** ;
Proc Factor data=chloud.watland method=ML;
    var AgPT EPT IBI Hab Rich;
run;

title1 'Principle components for Bio: AgPT EPT IBI Hab Rich';
title2 'Savannah River Basin ';
%plotit(data=prin, labelvar=sta_,
       Plotvars= prin2 prin1, color=black, colors=blue)
run;
*********************************************************;

****  PLS; 26 landscape metrics  ************************;
Options ps=255 ls=120;
Proc PLS data=Chloud.watland  cv=split(9)  cvtest(seed=12345);
     Model AgPT EPT IBI Hab Rich = Ag_hi Ag_slp_hi Ag_mod Ag_slp Ag_slp_mod Bar_slp_hi
         Bar_slp_mod Crop_slp Crop_slp_mod
         Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet
         Pct_wtr PwrPipTL Slope3 Sd_slp Mean_slp Slp_mod  Soil_er Strmden  TotRoad30 TotRoadWS;
         Output out=outWLT  predicted = yhat1-yhat5
                  yresidual = yres1-yres5
                  xresidual = xres1-xres26
                  xscore    = xscr
                  yscore    = yscr;
  run;

***********    Diagnostic Check Figures and VIP table ******************************:
axis1 label=(angle=270 rotate=90 "x score 2")
     major=(number=5) minor=none;
axis2 label=("X-score 1") minor=none;
```

```
Title "Biota(AgPT, EPT, IBI, Hab, Rich) and 26 Landscape Metrics ";
symbol1 v=dot c=blue i=none;
proc gplot data=outWLT;
      plot xscr2*xscr1=1
         / vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;
%let ifac = 1;
data pltanno; set outWLT;
      length text $ 5;
      retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
             color 'blue' style 'swissb';
       text=%str(Sta_); x=xscr&ifac; y=yscr&ifac;
      axis1 label=(angle=270 rotate=90 "Y score &ifac")
         major=(number=5) minor=none;
      axis2 label=("X-score &ifac") minor=none;
      symbol1 v=none i=none;
proc gplot data=outWLT;
      plot yscr&ifac*xscr&ifac=1
         / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;

data pltanno; set outWLT;
      length text $ 5;
      retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
             color 'blue' style 'swissb';
      text=%str(Sta_); x=xscr1; y=xscr2;
     axis1 label=(angle=270 rotate=90 "X score 2")
         major=(number=5) minor=none;
     axis2 label=("X-score 1") minor=none;
     symbol1 v=none i=none;
proc gplot data=outWLT;
      plot xscr2*xscr1=1
         / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;


ods output XWeights=xweights;
proc pls data=Chloud.watland nfac=2 details;
       Model AgPT EPT IBI Hab Rich = Ag_hi Ag_slp_hi Ag_mod Ag_slp Ag_slp_mod Bar_slp_hi
            Bar_slp_mod Crop_slp Crop_slp_mod
            Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet
            Pct_wtr PwrPipTL Slope3 Sd_slp Mean_slp  Slp_mod  Soil_er Strmden
            TotRoad30 TotRoadWS;
run;

proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
              out =xweights;
   data xweights; set xweights;
      rename col1=w1 col2=w2;
   data wt_anno; set xweights;
```

```
    length text $ 7;
    retain function 'label'
         position '5'
         hsys     '3'
         xsys     '2'
         ysys     '2'
         color    'blue'
         style    'swissb';
    text=%str(_name_); x=w1; y=w2;
run;

  axis1 label=(angle=270 rotate=90 "X weight 2")
      major=(number=5) minor=none;
  axis2 label=("X-weight 1") minor=none;
  symbol1 v=none i=none;
  proc gplot data=xweights;
    plot w2*w1=1 / anno=wt_anno vaxis=axis1
               haxis=axis2 frame cframe=white href=0 vref=0;
  run; quit;



*The following statements produce coefficients and the VIP ;
/*
/  Put coefficients, weights, and R**2's into data sets.
/-------------------------------------------------------*/
  ods listing close;
  ods output PercentVariation  = pctvar
          XWeights          = xweights
          CenScaleParms     = solution;
  proc pls data=Chloud.watland nfac=2 details;
   Model AgPT EPT IBI Hab Rich = Ag_hi Ag_slp_hi Ag_mod Ag_slp  Ag_slp_mod
            Bar_slp_hi  Bar_slp_mod Crop_slp Crop_slp_mod
            Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet
            Pct_wtr PwrPipTL Slope3 Sd_slp Mean_slp Slp_mod  Soil_er Strmden
            TotRoad30 TotRoadWS / solution;
  run;
  ods listing;

/*
/  Just reformat the coefficients.
/-------------------------------------------------------*/
  data solution; set solution;
    format EPT IBI Hab Rich 8.5;
    if (RowName = 'Intercept') then delete;
    rename RowName = Predictor AgPT       = AgPT;
    rename RowName = Predictor EPT     = EPT;
    rename RowName = Predictor IBI   = IBI ;
    rename RowName = Predictor Hab = Hab;
    rename RowName = Predictor Rich= Rich;
```

```
   run;

/*
/  Transpose weights and R**2's.
/-------------------------------------------------------*/
   data xweights; set xweights; _name_='W'||trim(left(_n_));
   data pctvar  ; set pctvar  ; _name_='R'||trim(left(_n_));
   proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
                  out =xweights;
   proc transpose data=pctvar(keep=_name_ CurrentYVariation)
                  out =pctvar;
   run;
   Proc Print data=pctvar;

/*
/  Sum the squared weights times the normalized R**2's.
/  The VIP is defined as the square root of this
/  weighted average times the number of predictors.
/-------------------------------------------------------*/

   proc sql;
     create table vip as
       select *
         from xweights left join pctvar(drop=_name_) on 1;
   data vip; set vip; keep _name_ vip;
     array w{2};
     array r{2};
     VIP = 0;
     do i = 1 to 2;
       VIP = VIP + r{i}*(w{i}**2)/sum(of r1-r2);
       end;
     VIP = sqrt(VIP * 26);
   data vipbpls; merge solution vip(drop=_name_);
   proc print data=vipbpls;
   run;

*Outlier ***************************************;
Proc PLS data=Chloud.watland nfac=1;
    Model AgPT EPT IBI Hab Rich = Ag_hi Ag_slp_hi Ag_mod Ag_slp  Ag_slp_mod Bar_slp_hi
        Bar_slp_mod Crop_slp Crop_slp_mod
        Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet
        Pct_wtr PwrPipTL Slope3 Sd_slp Mean_slp Slp_mod  Soil_er Strmden
        TotRoad30 TotRoadWS ;
  output out=stdres stdxsse=stdxsse stdysse=stdysse;
  data stdres; set stdres;
      xdist = sqrt(stdxsse);
      ydist = sqrt(stdysse);
   run;
Symbol1 i=needles v=dot c=blue;
Symbol2 i=needles v=dot c=red;
```

```
Symbol3 i=needles v=dot c=green;
Proc gplot data=stdres;
      plot xdist*Sta_=ecoregion / cframe = white ;
Proc gplot data=stdres;
      plot ydist*Sta_=ecoregion / cframe=white;
run;
****************************************************************************;

****    Refine (Prune) the above model and do the diagnostic checking by graphing   ********;
**  Use only the VIP with > 0.8 ****;
Proc PLS data=Chloud.watland cv=split(9) cvtest(seed=12345);
    Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
           Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
           Slope3 Sd_slp Mean_slp Soil_er Strmden ;
    Output out=outWLT  predicted = yhat1-yhat5
                 yresidual = yres1-yres5
                 xresidual = xres1-xres14
                 xscore    = xscr
                 yscore    = yscr;
  run;
axis1 label=(angle=270 rotate=90 "x score 2")
     major=(number=5) minor=none;
axis2 label=("X-score 1") minor=none;
Title "Biota(AgPT, ept, ibi, hab, rich) and 1
4 Landscape Metrics ";
symbol1 v=dot c=blue i=none;
proc gplot data=outWLT;
     plot xscr2*xscr1=1
       / vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;
%let ifac = 1;
data pltanno; set outWLT;
     length text $ 5;
     retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
          color 'blue' style 'swissb';
      text=%str(Sta_); x=xscr&ifac; y=yscr&ifac;
  axis1 label=(angle=270 rotate=90 "Y score &ifac")
       major=(number=5) minor=none;
  axis2 label=("X-score &ifac") minor=none;
  symbol1 v=none i=none;
  proc gplot data=outWLT;
     plot yscr&ifac*xscr&ifac=1
       / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;

data pltanno; set outWLT;
     length text $ 5;
     retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
          color 'blue' style 'swissb';
     text=%str(Sta_); x=xscr1; y=xscr2;
```

```
   axis1 label=(angle=270 rotate=90 "X score 2")
         major=(number=5) minor=none;
   axis2 label=("X score 1") minor=none    symbol1 v=none i=none;
   proc gplot data=outWLT;
      plot xscr2*xscr1=1
         / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;

ods output XWeights=xweights;
proc pls data=Chloud.watland nfac=2 details;
    Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
             Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
             Slope3 Sd_slp Mean_slp Soil_er Strmden ;
run;

proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
            out =xweights;
   data xweights; set xweights;
      rename col1=w1 col2=w2;
   data wt_anno; set xweights;
      length text $ 7;
      retain function 'label'
           position '5'
           hsys     '3'
           xsys     '2'
           ysys     '2'
           color    'blue'
           style    'swissb';
      text=%str(_name_); x=w1; y=w2;
run;

   axis1 label=(angle=270 rotate=90 "X weight 2")
         major=(number=5) minor=none;
   axis2 label=("X-weight 1") minor=none;
   symbol1 v=none i=none;
proc gplot data=xweights;
      plot w2*w1=1 / anno=wt_anno vaxis=axis1
               haxis=axis2 frame cframe=white href=0 vref=0;
run; quit;

*The following statements produce coefficients and the VIP ;
/*
/  Put coefficients, weights, and R**2's into data sets.
/-------------------------------------------------------*/
   ods listing close;
   ods output PercentVariation  = pctvar
            XWeights          = xweights
            CenScaleParms     = solution;
   proc pls data=Chloud.watland nfac=2 details;
     Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
```

```
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
         Slope3 Sd_slp Mean_slp Soil_er Strmden / solution;


   run;
   ods listing;

/*
/  Just reformat the coefficients.
/-------------------------------------------------------*/
   data solution; set solution;
      format EPT IBI Hab Rich 8.5;
      if (RowName = 'Intercept') then delete;
      rename RowName = Predictor AgPT       = AgPT;
      rename RowName = Predictor EPT     = EPT;
      rename RowName = Predictor IBI   = IBI ;
      rename RowName = Predictor Hab = Hab;
      rename RowName = Predictor Rich= Rich;
   run;

/*
/  Transpose weights and R**2's.
/-------------------------------------------------------*/
   data xweights; set xweights; _name_='W'||trim(left(_n_));
   data pctvar  ; set pctvar  ; _name_='R'||trim(left(_n_));
   proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
                out =xweights;
   proc transpose data=pctvar(keep=_name_ CurrentYVariation)
                out =pctvar;
   run;
   Proc Print data=pctvar;

/*
/  Sum the squared weights times the normalized R**2's.
/  The VIP is defined as the square root of this
/  weighted average times the number of predictors.
/-------------------------------------------------------*/

   proc sql;
      create table vip as
         select *
            from xweights left join pctvar(drop=_name_) on 1;
   data vip; set vip; keep _name_ vip;
      array w{2};
      array r{2};
      VIP = 0;
      do i = 1 to 2;
         VIP = VIP + r{i}*(w{i}**2)/sum(of r1-r2);
         end;
      VIP = sqrt(VIP * 14);
```

```
  data vipbpls; merge solution vip(drop=_name_);
  proc print data=vipbpls;
  run;

*Outlier *************************************;
Proc PLS data=Chloud.watland nfac=1;
     Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
        Slope3 Sd_slp Mean_slp Soil_er Strmden / solution;

    output out=stdres stdxsse=stdxsse stdysse=stdysse;
    data stdres; set stdres;
       xdist = sqrt(stdxsse);
       ydist = sqrt(stdysse);
  run;
Symbol1 i=needles v=dot c=blue;
Symbol2 i=needles v=dot c=red;
Symbol3 i=needles v=dot c=green;
Proc gplot data=stdres;
      plot xdist*Sta_=ecoregion / cframe = white ;
Proc gplot data=stdres;
      plot ydist*Sta_=ecoregion / cframe=white;
run;
*****************************************************************************;

**   Analyses by Ecoregion  ****************************************************;
***   Data contain missing and non missing biota  **********************************;

Proc PLS data=Chloud.watland cv=split(10) cvtest(seed=12345);
    Where Ecoregion='P';
    Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
            Slope3 sd_slp mean_slp Soil_er Strmden ;
    Output out=outWLT  predicted = yhat1-yhat5
                 yresidual = yres1-yres5
                 xresidual = xres1-xres14
                 xscore    = xscr
                 yscore    = yscr;
  run;

Proc PLS data=Chloud.watland cv=block(3) cvtest(seed=12345);
    Where Ecoregion='BR';
    Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
            Slope3 sd_slp mean_slp Soil_er Strmden ;
    Output out=outWLT  predicted = yhat1-yhat5
                 yresidual = yres1-yres5
                 xresidual = xres1-xres14
                 xscore    = xscr
                 yscore    = yscr;
```

```
  run;
Proc PLS data=Chloud.watland;* cv= cvtest(seed=12345);
   Where Ecoregion='CP';
   Model AgPT EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
          Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
          Slope3 sd_slp mean_slp Soil_er Strmden ;
   Output out=outWLT  predicted = yhat1-yhat5
               yresidual = yres1-yres5
               xresidual = xres1-xres14
               xscore    = xscr
               yscore    = yscr;
  run;

** Can do the diagnostic checking by plotting the figure followed by PLS above **********
```

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Appendix IV

SAS Statements for Predicting the Non-Measured Dependent Variables

```
** Create data sets that contain missing and non missing biota for prediction  ************;
**  24 Landscape vars                              *************************;

Data Chloud.BioNoMis;
    set Chloud.watland;
    if bio ne 'missing';
    if ecoregion='P';
    keep Sta_ ecoregion AgPT EPT IBI Hab Rich Ag_hi Ag_slp_hi Ag_mod Ag_slp  Ag_slp_mod
     Bar_slp_hi  Bar_slp_mod Crop_slp Crop_slp_mod
         Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet Pct_wtr PwrPipTL Slope3 sd_slp
     mean_slp Slp_mod  Soil_er Strmden TotRoad30 TotRoadWS;
run;
Proc Print data=Chloud.BioNoMis;
    var Sta_ AgPT EPT IBI Hab Rich;
run;
Data Chloud.BioMis;
    set Chloud.watland;
    if bio = 'missing';
    keep Sta_ Ecoregion Ag_hi Ag_slp_hi Ag_mod Ag_slp  Ag_slp_mod
            Bar_slp_hi  Bar_slp_mod Crop_slp Crop_slp_mod
            Past_slp Pct_bar  Pct_crop Pct_for Pct_past Pct_urb Pct_wet
            Pct_wtr PwrPipTL Slope3 sd_slp mean_slp Slp_mod  Soil_er Strmden
            TotRoad30 TotRoadWS;
run;
Proc Print data=Chloud.BioMis;
    Var Sta_ Ecoregion;
run;
Data Chloud.Bio;
    set Chloud.watland;
    ObsAgPT= AgPT;
    Obsept = EPT;
    Obsibi = IBI;
    ObsHab = Hab;
    ObsRich= Rich;
    if bio = 'missing';
    keep Ecoregion Sta_ ObsAgPT Obsept Obsibi ObsHab ObsRich;
run;
```

```
Proc Print data=Chloud.Bio;
run;

************  Prediction for the Piedmont only; considering VIP           ***************;
**  Data without missing bio data, no AgPT, sd_slp and mean_slp ; Oct 10 2002;  nLS=12 ***;

Proc PLS data=Chloud.BioNoMis cv=Block(14) cvtest(seed=12345); ** split(15) Block(14,30,45) **;
    Model EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
            Slope3 Soil_er Strmden;
    Output out=outWLT  predicted = yhat1-yhat4

         yresidual = yres1-yres4

         xresidual = xres1-xres12
                         xscore    = xscr
                      yscore    = yscr;
run;
Proc Print data=outWLT;
    Var Sta_ Ecoregion EPT yhat1 IBI yhat2 Hab yhat3 Rich Yhat4;
run;
Symbol1 v=dot i=none c=blue;
Proc Gplot data=outWLT;
    Plot EPT*Yhat1 =1;
    Plot IBI*Yhat2 =1;
    Plot Hab*Yhat3=1;
    Plot Rich*Yhat4=1;
run;

%let ifac = 1;
data pltanno; set outWLT;
     length text $ 5;
     retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
          color 'blue' style 'swissb';
     text=%str(Sta_); x=xscr&ifac; y=yscr&ifac;
     axis1 label=(angle=270 rotate=90 "Y score &ifac")
       major=(number=5) minor=none;
     axis2 label=("X-score &ifac") minor=none;
     symbol1 v=none i=none;
proc gplot data=outWLT;
     plot yscr&ifac*xscr&ifac=1
       / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;
data pltanno; set outWLT;
     length text $ 5;
     retain function 'label' position '5' hsys '3' xsys '2' ysys '2'
          color 'blue' style 'swissb';
     text=%str(Sta_); x=xscr1; y=xscr2;
     axis1 label=(angle=270 rotate=90 "X score 2")
       major=(number=5) minor=none;
```

```
    axis2 label=("X-score 1") minor=none;
    symbol1 v=none i=none;
proc gplot data=outWLT;
    plot xscr2*xscr1=1
      / anno=pltanno vaxis=axis1 haxis=axis2 frame cframe=white href=0 vref=0;
run;
ods output XWeights=xweights;
proc pls data=Chloud.BioNoMis nfac=2 details;
   Model EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
              Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
              Slope3 Soil_er Strmden;
run;
Proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
             out =xweights;
  data xweights; set xweights;
    rename col1=w1 col2=w2;
  data wt_anno; set xweights;
    length text $ 7;
    retain function 'label'
         position '5'
         hsys     '3'
         xsys     '2'
         ysys     '2'
         color    'blue'
         style    'swissb';
    text=%str(_name_); x=w1; y=w2;
  run;

  axis1 label=(angle=270 rotate=90 "X weight 2")
      major=(number=5) minor=none;
  axis2 label=("X-weight 1") minor=none;
  symbol1 v=none i=none;
  proc gplot data=xweights;
    plot w2*w1=1 / anno=wt_anno vaxis=axis1
               haxis=axis2 frame cframe=white href=0 vref=0;
  run; quit;

***** The following statements produce coefficients and the VIP ;
/*
/  Put coefficients, weights, and R**2's into data sets.
/-------------------------------------------------------*/
  ods listing close;
  ods output PercentVariation  = pctvar
            XWeights         = xweights
            CenScaleParms     = solution;
  proc pls data=Chloud.BioNomis nfac=8 details;
   Model EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
              Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
              Slope3 Soil_er Strmden / solution;
  run;
```

```
   ods listing;

/*
/  Just reformat the coefficients.
/-------------------------------------------------------*/
   data solution; set solution;
      format EPT IBI Hab Rich 8.5;
      if (RowName = 'Intercept') then delete;
      rename RowName = Predictor EPT     = EPT;
      rename RowName = Predictor IBI   = IBI ;
      rename RowName = Predictor Hab = Hab;
      rename RowName = Predictor Rich= Rich;
   run;

/*
/  Transpose weights and R**2's.
/-------------------------------------------------------*/
   data xweights; set xweights; _name_='W'||trim(left(_n_));
   data pctvar  ; set pctvar  ; _name_='R'||trim(left(_n_));
   proc transpose data=xweights(drop=NumberOfFactors InnerRegCoef)
               out =xweights;
   proc transpose data=pctvar(keep=_name_ CurrentYVariation)
               out =pctvar;
   run;

/*
/  Sum the squared weights times the normalized R**2's.
/  The VIP is defined as the square root of this
/  weighted average times the number of predictors.
/-------------------------------------------------------*/
options ps=255 ls=120;
Title ' VIP for the piedmont, nonmissing: nLS=12';
   proc sql;
      create table vip as
         select *
            from xweights left join pctvar(drop=_name_) on 1;
   data vip; set vip; keep _name_ vip;
      array w{8};
      array r{8};
      VIP = 0;
      do i = 1 to 8;
         VIP = VIP + r{i}*(w{i}**2)/sum(of r1-r8);
         end;
      VIP = sqrt(VIP * 12);
   data vipbpls; merge solution vip(drop=_name_);
   proc print data=vipbpls;
   run;

*Outlier ****************************************;
Proc PLS data=Chloud.BioNoMis nfac=1;
```

```
    Model EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
            Slope3 Soil_er Strmden;
    output out=stdres stdxsse=stdxsse stdysse=stdysse;
    data stdres; set stdres;
        xdist = sqrt(stdxsse);
        ydist = sqrt(stdysse);
  run;
Symbol1 i=needles v=dot c=blue;
Proc gplot data=stdres;
      plot xdist*Sta_=1 / cframe = white ;
Proc gplot data=stdres;
      plot ydist*Sta_=1 / cframe=white;
run;
**************************************************************;

****  Prediction of the missing bio data Oct 2002,  nLS = 12 ****;
Title ' Predicted for missing observations, Piedmont  ';
Options ps=255 ls=180;
Data Chloud.all; Set Chloud.BioNoMis Chloud.BioMis;
run;
Proc PLS data=Chloud.all cv=block(14) cvtest(seed=12345);
    Model EPT IBI Hab Rich = Ag_mod Ag_slp  Ag_slp_mod
            Crop_slp Crop_slp_mod Past_slp Pct_bar Pct_for Pct_past
            Slope3 Soil_er Strmden;
    Output out=Pred  p=Predept Predibi PredHab PredRich;
run;
Proc Print data=pred;
    Where (Sta_ in ('S04','S114','S176','S177','S207','S211','S224','S231',
                'S236','S238','S31','S44','S45','S48','S51','S57','S89','S97'));
    var Ecoregion Sta_ PredEPT PredIBI PredHab PredRich;
run;
***************  End of prediction   ************************;
```

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Environmental Protection
Agency

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